CHEMICAL ANALYSIS

Volume I:	*The Analytical Chemistry of Industrial Poisons, Hazards and Solvents* By Morris B. Jacobs
Volume II:	*Chromatographic Adsorption Analysis* By Harold H. Strain
Volume III:	*Colorimetric Determination of Traces of Metals* By E. B. Sandell
Volume IV:	*Organic Reagents Used in Gravimetric and Volumetric Analysis* By John F. Flagg
Volume V:	*Aquametry. Application of the Karl Fischer Reagent to Quantitative Analyses Involving Water* By John Mitchell, Jr., and Donald Milton Smith
Volume VI:	*Analytical Instruments and Technique* By R. H. Muller

Other volumes in preparation

CHEMICAL ANALYSIS

A SERIES OF MONOGRAPHS ON
ANALYTICAL CHEMISTRY AND ITS APPLICATIONS

Volume V

AQUAMETRY

by JOHN MITCHELL, JR., M.S.
and DONALD MILTON SMITH, Ph.D.

INTERSCIENCE PUBLISHERS, INC., NEW YORK
INTERSCIENCE PUBLISHERS LTD., LONDON

AQUAMETRY

Application of the Karl Fischer Reagent to Quantitative Analyses Involving Water

JOHN MITCHELL, Jr., M.S., *and*
DONALD MILTON SMITH, Ph.D.
Ammonia Department, E. I. du Pont de Nemours & Co., Inc.
Wilmington, Delaware

1948

INTERSCIENCE PUBLISHERS, INC., NEW YORK
INTERSCIENCE PUBLISHERS LTD., LONDON

INTERSCIENCE PUBLISHERS, INC.
215 Fourth Avenue, New York 3, N.Y.

PRINTED IN THE UNITED STATES OF AMERICA
BY MACK PRINTING COMPANY, EASTON, PA.
COMPOSITION BY WESTCOTT & THOMSON, INC., PHILADELPHIA, PA.

PREFACE

Up to the present time no generic name has been available for the analytical process of water measurement that has become so essential in the evaluation of all types of materials. The authors have coined the term "Aquametry" to represent precisely this important phase of analytical chemistry.

Since its discovery in 1935, the Karl Fischer reagent has become highly useful in the determination of the moisture associated with a wide variety of compounds and reactions, both organic and inorganic. The authors have attempted to evaluate critically the rapidly increasing literature on the subject up to the early months of 1947. It should be emphasized, perhaps, that the mechanisms underlying many of the reactions still are incompletely known.

The principal objective in preparing this book has been to make available to analytical chemists the various applications of the Karl Fischer reagent. All procedures based on the reagent have been discussed in detail so that anyone wishing to employ the methods should have little difficulty in reproducing the work described, provided the instructions are followed exactly.

Although it was not intended to present a comprehensive treatise on the determination of water, it has seemed advisable to review most of the techniques in order to determine the relative merits of the methods based on the Karl Fischer reagent. Where it has appeared advisable, the details of certain of these other procedures have been given. This has applied, in particular, to the chemical methods for the determination of free water. Most of this information is included in Chapter I.

In Chapter II the authors have departed from usual practice. This chapter presents a rather complete summary of the analytical applications of the Karl Fischer reagent. It is intended as a reference chapter and is situated in this part of the book in order to be readily accessible as a laboratory manual.

Most of the reported difficulties in applying the visual endpoint to the Fischer reagent titration have involved the color to be used. In general there is a tendency to undertitrate, that is, to choose as the endpoint the indefinite color change from canary to chromate yellow. After a little practice, the endpoint is observed as a sharp, accurately reproducible change from chromate yellow to the color of unused iodine. An excess of 0.1 to 0.2 milliliter of Fischer reagent results in a further marked change, giving a very deep brown solution. Further details regarding this technique are included in Chapter IV.

The book includes the results of several years' experience in the authors' laboratory and contains much previously unpublished data on determinations of free water and on quantitative analyses for organic functional groups. The final chapter suggests several possible subjects for future study.

The authors are greatly indebted to W. M. D. Bryant for his many contributions, to Walter Hawkins for his aid in carrying out most of the recent experimental program, and to C. W. Hammond, who, with the help of E. J. Mulderick, prepared all of the photographs related to studies in the authors' laboratory. The authors also wish to thank C. E. Ashby, W. L. Autman, Alberta Barta, and A. N. Oemler for their help and suggestions in some of the experimental work and E. Rosalee Mitchell for her aid in reading and correcting the manuscript.

Wilmington, Delaware
October, 1948

JOHN MITCHELL, JR.
DONALD MILTON SMITH

CONTENTS

PART ONE

Specific Quantitative Determination of Water by Karl Fischer Reagent. Reactions of Inorganic Compounds

PART TWO

Organic Reactions Involving Liberation or Consumption of Water. Application of Karl Fischer Reagent to Quantitative Determination of Organic Functional Groups

PART ONE

Specific Quantitative Determination of Water by Karl Fischer Reagent. Reactions of Inorganic Compounds.

CHAPTER I

Methods for the Determination of Water

Since the determination of water must be included in nearly every complete analysis, methods for its estimation have been the subject of a great many analytical investigations. Although a large number of procedures have been developed for application to specific materials, few of the techniques have proved generally applicable. Separation (especially oven drying, distillation, or absorption) has been the basis of most routine methods. Procedures based on chemical reactions or measurements of physical properties usually have been employed for special cases.

A. Oven Drying

Oven drying, probably the oldest method, undoubtedly has been the most widely used technique for the determination of moisture. Ordinarily a gravimetric measurement of weight loss was made at temperatures near the boiling point of water. In general this was a time consuming process which did not differentiate between water and other volatile matter; it was useless for materials which were thermally unstable and was limited by humidity conditions in the oven. In specific instances many of these objections have been minimized by the use of circulating hot air or by the use of vacuum ovens; for example, the time required to dry roughages[1] and tung fruit[2] was reduced considerably when a special drying machine, employing a hot air circulator, was substituted for the gravity convection oven. Other empirical methods employing oven drying have included those involving the estimation of moisture in products from sulfite waste liquors,[3] and in starches,[4] potato flakes,[5] hops,[6] drugs,[7]

---

[1] Monroe, C. F., and Perkins, A. E., *J. Dairy Sci.*, **22**, 37–9 (1939).

[2] Freeman, A. F., Pack, F. C., and McKinney, R. S., *Oil & Soap*, **20**, 203–4 (1943).

[3] Roll, F., and Arland, A., *Giesserei*, **31**, 110–13 (1944); *C.A.*, **39**, 4823 (1945).

[4] Porter, W. L., and Willits, C. D., *J. Assoc. Official Agr. Chem.*, **27**, 179–94 (1944).

and barley.[8] Göhde[9] developed an apparatus for the determination of moisture in cellulose derivatives consisting of an electrically heated cabinet with built-in automatic temperature regulator, air circulator, and projection reading balance. With this equipment he demonstrated that cellulose, wood pulp, and paper could be dried to constant weight in from 20 to 70 minutes, a fraction of the time required by normal oven drying. This general scheme has been incorporated in commercial semiautomatic instruments. Brock[9a] developed a novel moisture tester employing infrared radiation. This source of heat was found to have the unique property of driving out the water vapor from solids and some liquids, although the temperature in the samples was maintained at 70°C. or below.

For the rapid determination of moisture in oils, the water has been volatilized and the residue weighed.[10] Similar techniques have been employed for the estimation of moisture in butter and cheese[11] and in whey products.[12]

B. Distillation

Distillation methods have been used widely in the soap, oil, and related industries. In general, these procedures depended on a heterogeneous azeotropic distillation with hydrocarbons or organic halides in which the water, separating as a separate phase in the distillate, was measured volumetrically. An excellent historical survey of distillation methods has been presented by Cleland and Fetzer[13] who noted that the hydrocarbons most commonly employed were benzene, toluene, xylene, and various petroleum fractions, while the halide most often used was carbon tetrachloride. Tetrachloroethane has been found useful.[14] Johnson[15] recommended chloroform dis-

---

[5] Schmalfuss, H., and Müller, H., *Z. Spiritusind.*, 66, 2 (1943); *C.A.*, 39, 3373 (1945).

[6] Nowak, G., and Enders, C., *Tageszlg. Brau.*, 36, 27–8 (1938); *C.A.*, 39, 3621 (1945).

[7] Golovko, D. N., *Farmatsiya,* 1944, 29–32; *C.A.*, 39, 3395 (1945).

[8] Bishop, L. R., *J. Inst. Brewing,* 50, 141–5 (1944).

[9] Göhde, K., *Papier-Fabr.*, 37, 320–4 (1939).

[9a] Brock, R. B., *Chemistry & Industry,* 1947, 299–302.

[10] Grant, J., *Chemist-Analyst,* 29, 79–80 (1940).

[11] Olsson, T., *Svenska Mejeritidn.*, No. 31 (1941); *Dairy Sci. Abstracts,* 5, 102 (1943).

[12] Olsson, T., *Ibid.*, No. 6 (1944); *C.A.*, 39, 2350 (1945).

[13] Cleland, J. E., and Fetzer, W. R., *Ind. Eng. Chem., Anal. Ed.,* 14, 124–7 (1942).

[14] Phillips, E., and Enas, J. D., *J. Assoc. Official Agr. Chem.*, 27, 442–6 (1944).

[15] Johnson, F. B., *Can. Chem. & Process Inds.*, 1943, 100, 102.

tillation for the analysis of dehydrated vegetables. One of the objectionable features of distillation techniques for soaps, that of excessive foaming, was eliminated by Trusler[16] who precipitated the troublesome compounds with anhydrous barium chloride. Atkins[17] found that ternary homogeneous azeotropes were sometimes applicable in cases where heterogeneous binaries were inconvenient or gave unsatisfactory results. Entraining agents which dissolved or replaced water in the sample or in which the sample was soluble tended to be helpful. Recommended azeotropes[17] included these systems: benzene–ethanol–water (B.P. 64.9°C., 7.4 per cent water), carbon tetrachloride–methyl ethyl ketone–water (B.P. 65.7°C., 3.0 per cent water), and carbon tetrachloride–ethanol–water (B.P. 61.8°C., 3.4 per cent water). A procedure employing a ternary system suffered from the disadvantage of requiring a precise fractional distillation if water content was to be estimated from the total volume of azeotrope recovered.

C. Absorption

Absorption techniques usually were based on the evolution of water at elevated temperatures into a stream of inert gas by which it was carried into a tared tower containing an active desiccant. These methods have been used widely in the evaluation of insulating oils and cellulose products. Anhydrous magnesium perchlorate (Dehydrite) and calcium sulfate (Drierite) appeared to be favored as absorption agents,[18,19] although phosphorus pentoxide[20] and calcium chloride[21–23] also were used for this purpose. All absorption techniques were subject to possible error from other volatile constituents; they were time consuming and required rather elaborate apparatus. Evans and his coworkers[19] minimized the errors resulting from absorption on the desiccant of such substances as volatile hydrocarbon components of oils. The material to be analyzed for moisture was placed in a bulb, heated at 105°C., and the

---

[16] Trusler, R. B., *Oil & Soap,* **16,** 239–41 (1939).

[17] Atkins, W. R. G., *Nature,* **150,** 449 (1943).

[18] Reiner, S., *Electrotech. Z.* **46,** 1447–8 (1925).

[19] Evans, R. N., Davenport, J. E., and Revukas, A. J., *Ind. Eng. Chem., Anal. Ed.,* **11,** 553–5 (1939); **13,** 589–92 (1941).

[20] Clifford, C. W. J., *Ind. Eng. Chem.,* **13,** 628–31 (1921).

[21] Fulweiler, W. H., *Proc. Am. Soc. Testing Materials,* **25, I,** 416–23 (1925).

[22] Parsons, L. B., and Holmberg, C. O., *Oil & Soap,* **14,** 239 (1937).

[23] Joyner, N. T., and Rini, S., *Ibid.,* **16,** 233–6 (1939).

evolved water and hydrocarbon vapors were passed through an Apiezon oil scrubber, which preferentially removed a portion of the hydrocarbons. The remaining vapors were passed through a cold trap at the temperature of solid carbon dioxide (—78°C.) where moisture and oils condensed while other volatile constituents, such as hydrogen, methane, and carbon monoxide, passed through. The condensed vapors were carried into a combustion furnace where the hydrocarbon was oxidized to carbon dioxide and water. This water of reaction together with original free water was absorbed on Dehydrite and the carbon dioxide, on Ascarite. The net or initial moisture content was then calculated from an empirical correction derived from the quantity of carbon dioxide found.

Less involved absorption methods, applied to the analysis of glycerol, were based on distribution of the sample on ignited asbestos for exposure to phosphorus pentoxide at a pressure of 10 to 15 mm.[24,25] or vacuum distillation with subsequent absorption of the volatilized moisture by Dehydrite.[25] Day and Pease[26] treated a complex solution of alcohols, aldehydes, acids, and peroxides, resulting from hydrocarbon oxidations, with a weighed excess (two- to threefold) of powdered anhydrous copper sulfate. The solid was recovered, washed with liquid butane to remove adhering organics and, after suction drying, weighed. The increase in weight was a measure of the water content of the sample. (An error of only 1 per cent was found on methanol and methanol–paraldehyde solutions containing 20 per cent water.) In some cases moisture has been evolved from loosely packed solids by circulating hot dry air and absorbing the effluent gases in tared drying tubes.[27]

An extensive investigation was made by McLean and Kohman[28] on the effect of liquid impregnants in the determination of moisture in electrical grade paper by a vacuum evaporation-absorption technique. They demonstrated that virtually all of the moisture in unimpregnated paper was removed in 2 to 3 hours at a temperature of 120°C. and a pressure of less than 1 mm. of mercury. When the

---

[24] Rojahn, C. A., *Z. anal. Chem.*, 58, 433–42 (1919).

[25] Spaeth, C. P., and Hutchison, G. F., *Ind. Eng. Chem., Anal. Ed.*, 8, 29–32 (1936).

[26] Day, R. A., Jr., and Pease, R. N., *J. Am. Chem. Soc.*, 61, 524–5 (1939).

[27] Kuentzel, W. E., *Ibid.*, 51, 3560–1 (1929).

[28] McLean, D. A., and Kohman, G. T., *J. Franklin Inst.*, 228, 223–34 (1940).

paper was immersed in an impregnant of low vapor pressure, however, no more than half of the moisture was removed after 4 hours at 120°C.; a temperature of 180°C. was required before water evolution was essentially complete.* As a result of this study they specified that where possible most of the excess impregnant be permitted to drain off before suspending the paper in the sample vessel. After being connected to the absorption train (trap and tared phosphorus pentoxide absorption tube), the sample was allowed to reach a bath temperature of about 150°C. before the pressure was reduced. At this temperature all wax impregnants became completely molten so that when evacuation was begun, the sudden rush of water vapor and dissolved gases tended to purge the paper of additional impregnant, which condensed and collected at the bottom of the vessel. Under these conditions an evacuation period of 4 hours at 150°C. usually was sufficient for essentially complete removal of the water from 10 gram samples containing up to 5 per cent moisture.

D. Miscellaneous Methods

There are numerous methods of limited applicability based on measurement of some physical property. Specific gravity has been used widely for the analysis of some alcohols, based on the published data of Osborne, McKelvey, and Bearce,[29] Bosart and Snoddy,[30] and Doroshevskii.[31] Shnidman[32] employed a gravity tech-

---

* The difficulty of drying paper in the presence of an impregnant was explained according to their theory by "trapping" of the moisture by surface tension forces. In drying paper, such as that used in electrical work, the first moisture evolved would sweep out most of the entrapped gas, after which the water vapor in the pores of the paper would be subjected to a pressure of $P_1 + (2\lambda/R)$ equal to P_2, where P_1 is the pressure in the surrounding medium, λ is the surface tension of the impregnant, R is the effective pore radius of the paper, and P_2 is the pressure within the pores, corresponding to a point on the equilibrium desorption isotherm for the particular system. On the basis of this theory McLean and Kohman[28] concluded that when moist paper is evacuated under a liquid of low vapor pressure, its final equilibrium moisture content will not correspond to P_1 but to a pressure $2\lambda/R$ greater than this.

[29] Osborne, N. S., McKelvey, E. C., and Bearce, H. W., *Bull. Bureau of Standards*, **9**, 327 (1913).

[30] Bosart, L. W., and Snoddy, A. O., *Ind. Eng. Chem.*, **19**, 506–10 (1927).

[31] Doroshevskii, A. G., *J. Russ. Phys. Chem. Soc.*, **40**, 908 (1908); **41**, 958, 977, 1428 (1909).

[32] Shnidman, L., *Ind. Eng. Chem., Anal. Ed.*, **15**, 430–3 (1943).

nique for sulfur paste. Electrical conductivity,[33-35] refractive index,[31, 36] and viscosity[37] have all been employed. Cameron and his coworkers,[38] studying corn, compared the use of refractive index with oven drying, gravity separation, and "Yardman's Grade" (an empirical sampling method) for the estimation of moisture content which was used as a criterion of maturity. Critical solution temperatures have been employed by Robertson[39] (dicyclohexyl), Wood and Neale[40] (aniline), and Jones and Amstell[41] (cyclohexane). Centrifugation for suspended moisture[42] and measurements of heat of hydration[43] or relative humidity[19, 44] also have been recommended.

For hydrocarbon gases procedures have been described based on dew point,[45] heat of dilution in sulfuric acid,[46] pressure differential after exposure to lithium chloride,[47] near infrared absorption spectra,[48] and condensation into a tared cold-trap where the water was weighed[49] or into a buret where the volume change was recorded.[50] The color change of cobaltous bromide on hydration has been made the basis of both a visual[51] and a photoelectric[52] method for the estimation of moisture in fuel gases. In the latter procedure Pfister and Kerley[52] collected the water vapor in a solution of cobaltous

---

[33] Schmidt, M. R., and Jones, H. C., *Am. Chem. J.*, 42, 37–41 (1909).

[34] Kameyama, N., and Semba, T., *J. Soc. Chem. Ind., Japan,* 30, 10–14 (1927).

[35] Lund, H., and Bjerrum, J., *Ber.*, 64, 210–13 (1931).

[36] Hoyt, L. F., *Ind. Eng. Chem.*, 26, 329–32 (1934).

[37] Hartley, H., and Raikes, H. R., *J. Chem. Soc.*, 127, 524–7 (1925).

[38] Cameron, J. K., Dykstra, K. G., and Fix, J. E., *Food Industries,* July, 1942.

[39] Robertson, G. R., *Ind. Eng. Chem., Anal. Ed.*, 15, 451–2 (1943).

[40] Wood, C. E., and Neale, A. E. T., *J. Inst. Petroleum Tech.*, 11, 471–4 (1925).

[41] Jones, D. C., and Amstell, J., *J. Chem. Soc.*, 1930, 1316–23.

[42] Rosenthal, *Chem.-Ztg.*, 33, 1259 (1909).

[43] Pflug, H., *Ibid.*, 51, 717–8 (1918).

[44] Dunmore, F. W., *J. Research Natl. Bur. Standards*, 23, 701–14 (1939).

[45] Deaton, W. M., and Frost, E. M., Jr., *U. S. Bur. Mines, Repts. Investigations,* No. 3399, 4 pp. (May, 1938).

[46] Haywood, F. W., Bosanquet, C. H., and Pearson, J. L., British Patent 491,154 (1938).

[47] Evans, R. N., and Davenport, J. E., *Ind. Eng. Chem., Anal. Ed.*, 14, 732–3 (1942).

[48] Todd, F. C., and Gauger, A. W., *Proc. Am. Soc. Testing Materials,* 41, 1134–51 (1941).

[49] Perry, C. W., *Ind. Eng. Chem., Anal. Ed.*, 10, 513–4 (1938).

[50] Grandval, J. H., *Chaleur et ind.*, 26, 51–5 (1945).

[51] Natural Gasoline Assoc., *Natl. Petroleum News,* 24, 38 (April 6, 1932).

[52] Pfister, R. J., and Kerley, D. J., *Am. Soc. Testing Materials Bull.*, 127, 17–22 (1944).

bromide in butanol and then measured the transmission at 660 microns. Moisture in aniline has been estimated from the cloud point with rapeseed oil[53, 54] or a cottonseed oil–heavy mineral oil composition[55] and in furfural, with a hexanol–cottonseed oil reagent.[56] The equilibrium humidity has been correlated with moisture content of textiles[57] and of dehydrated foods.[58] The boiling point of the melt at reduced pressure has been related to moisture in ammonium nitrate.[59]

An interesting polarimetric method has been proposed by Toennies and Elliott[60] for the determination of water in acetic acid based on hydrolysis of acetic anhydride in the presence of perchloric acid catalyst followed by reaction of the excess anhydride with *d*-camphoric acid:

$$(CH_3CO)_2O + C_8H_{14}(COOH)_2 \longrightarrow 2\ CH_3COOH + C_8H_{14}(CO)_2O \qquad (1)$$

The decrease in optical rotation of a known quantity of camphoric acid became a measure of unused anhydride from which original water could be calculated. The accuracy of the method based on analyses of acetic acid containing 0.1 and 0.6 per cent water was found to be ±0.001 and ±0.008 per cent, respectively.

The last several years have seen the development of apparatus suitable for the rapid measurement of electrical properties which are a function of water concentration. In most cases these instruments measure electrical conductivity, resistance, or dielectric constant. Conductivity measurements have been employed in the estimation of moisture in wood,[61-64] textiles,[65] granular materi-

---

[53] Jansen, J. D., and Schut, W., *Chem. Weekblad,* 20, 657–8 (1923).

[54] Griswold, J., *Ind. Eng. Chem., Anal. Ed.,* 12, 89–90 (1940).

[55] Seaman, W., Norton, A. R., and Hugonet, J., Jr., *Ibid.,* 15, 322–4 (1943).

[56] Griswold, J., Klecka, M. E., and West, R. V. D., *Ibid.,* 18, 696–8 (1946).

[57] Matthew, J. A., *J. Textile Inst.,* 27, T219–28 (1936).

[58] Vincent, J. F., and Bristol, K. E., *Ind. Eng. Chem., Anal. Ed.,* 17, 465–6 (1945).

[59] Zil'berman, D. E., *Zavodskaya Lab.,* 11, 108–9 (1945); *C.A.,* 39, 4025 (1945).

[60] Toennies, G., and Elliott, M., *J. Am. Chem. Soc.,* 59, 902–6 (1937).

[61] Suits, C. G., and Dunlop, M. E., *Southern Lumberman,* 140, No. 1782, 68–70 (1930).

[62] Limbrick, C. B., United States Patent 1,890,545 (1942).

[63] Store, A. O., Canadian Patent 362,927 (1936).

[64] Bakman, L., *Aviapromyshlennost,* No. 2, 76–8 (1940); *C.A.,* 34, 4616 (1940).

[65] Seigenheim, M., United States Patent 2,189,352 (1940).

als,[66–72] and, to a limited extent, in gases based on absorption of the water in an organic solvent on which the conductance was determined.[73] Electrical resistance also has been applied widely, to the determination of water in textiles,[74, 75] ceramics,[76] paper,[77] tobacco,[78] and petroleum oils.[79] The large difference in the dielectric constant of water and many commercial solids has been noted and instruments have been designed relating this property to the moisture contents of textiles,[80, 81] grain and granular materials,[82–86] and some drugs.[87] Solvent mixtures such as dioxane with benzene, carbon tetrachloride, or ethanol have been used to extract moisture from solids whose moisture content then was estimated from the dielectric constant of the extract.[88] For similar applications, capacitance measurements have been used.[81,89] A critical examination was made by Cook, Hopkins, and Geddes[90] of several types of instruments adapted to the estimation of moisture in grain. These instruments usually required a relatively large sample in which the moisture had to be distributed evenly; they usually were unsuited to materials of low water content (below 8 to 10 per cent[8, 64]).

Other novel equipment for the determination of moisture included an apparatus for generating high frequency mechanical

---

[66] Stein, F. W., United States Patent 2,251,641 (1941).

[67] McIlvaine, R. L., German Patent 483,580 (1926).

[68] Heppenstall, T. E., United States Patent 1,826,247 (1931).

[69] Pusrin, G. B., *Mühlenlab.*, **3**, 69–74 (1933).

[70] Dawson, J., British Patent 454,438 (1936).

[71] Stevens, R. L., United States Patent 2,343,340 (1944).

[72] Groves, L. G., and King, J., *J. Soc. Chem. Ind.*, **65**, 320–4 (1946).

[73] Komarov, V. A., Russian Patent 51,904 (1937).

[74] Field, J. F., British Patents 409,338; 412,565 (1934).

[75] Jones, E. H., *J. Sci. Instruments*, **17**, 55–62 (1940).

[76] Makarin, S. N., Russian Patent 46,723 (1936).

[77] Jones, E. H., British Patent 516,379 (1940).

[78] Kudin, I. P., *Tabak*, **9**, No. 5, 21–3 (1939).

[79] O'Donnell, J. L., United States Patent 2,082,213 (1937).

[80] Mankasch, E., *Zavodskaya Lab.*, **8**, 471–4 (1939).

[81] Walter, H., German Patent 539,026 (1930).

[82] Guryanova, E. N., *Zavodskaya Lab.*, **10**, 569 (1941).

[83] Hartman, S. H., British Patent 440,966 (1936).

[84] Montlaur, L., *Compt. rend. acad. agr. France*, **16**, 931–7 (1930).

[85] Frankfurter Forschungsinstitut für Getreidechemie, German Patent 524,510 (1927).

[86] Eyer, B. F., United States Patent 2,043,241 (1936).

[87] van Steenbergen, B., *Pharm. Weekblad*, **73**, 244–73 (1936).

[88] Ebert, L., and Waldschmidt, E., German Patent 616,493 (1936).

[89] Bartlett, H. W., United States Patent 2,266,114 (1941).

[90] Cook, W. H., Hopkins, J. W., and Geddes, W. F., *Can. J. Research*, **11**, 409–47, 547–63 (1934).

oscillations where the rate at which the oscillations were transmitted through the material were related to moisture content,[91,92] an instrument for generating sound waves on one side of a material and receiving them on the other,[93] and a device in which an initially dry gas was permitted to come to equilibrium with a solid sample, after which the thermal conductivity of the gas was measured.[94]

A wide variety of chemical methods have been employed for the determination of water but these were often qualitative, being subject to many types of interference and usually restricted to the detection of very small amounts of water.

The discussion of some of the chemical methods is more descriptive than the previous survey of physical techniques. It seems desirable to state clearly the principles employed (physical methods usually are self-explanatory), and the limitations of the procedures when applied as general techniques. Reference is made to certain of these procedures in later chapters.

Graefe[95] measured the hydrogen evolved after treatment of the material with sodium:

$$2\,H_2O + 2\,Na \longrightarrow 2\,NaOH + H_2 \qquad (2)$$

Calcium carbide has been used because of the great ease with which it evolves acetylene when exposed to the action of water:

$$CaC_2 + 2\,H_2O \longrightarrow HC{\equiv}CH + Ca(OH)_2 \qquad (3)$$

The acetylene has been measured gasometrically,[96-98] colorimetrically as cuprous acetylide,[37,99] and gravimetrically after ignition of the cuprous acetylide to cupric oxide.[100] Several patents have been issued describing methods employing calcium carbide for the estimation of moisture: by weight loss after evolution of the acetylene formed in the reaction,[101] by measurment of flame intensity during combustion,[102] or by relation to oxygen requirements during combustion.[103]

---

[91] Warren, A., German Patent 670,261 (1939).
[92] The Foxboro Co., British Patent 444,056 (1936).
[93] Brough, A. F., and Puleston, P. R., British Patent 468,005 (1937).
[94] Grüss, H., German Patent 648,275 (1937).
[95] Graefe, E., *Petroleum*, 1, 813–17 (1906).
[96] Boller, W., *Chem.-Ztg.*, 50, 537–8 (1926).
[97] Roberts, R. W., and Fraser, A., *J. Soc. Chem. Ind.*, 29, 1977–8 (1910).
[98] Delmhorst, W. J., United States Patent 2,359,831 (1944).
[99] Weaver, E. R., *J. Am. Chem. Soc.*, 36, 2462–8 (1914).
[100] Vtorova, E. I., *Sintet. Kauchuk*, 5, 29–31 (1936).
[101] Parks, R. Q., United States Patent 2,362,396 (1944).
[102] Chopin, M., United States Patent 2,281,182 (1942).
[103] Naklonov, V. A., Russian Patent 52,306 (1937).

The use of calcium hydride in the analysis for water in various organic compounds[104–108] was based on the reaction:

$$CaH_2 + 2\,H_2O \longrightarrow Ca(OH)_2 + 2\,H_2 \qquad (4)$$

The evolved hydrogen was measured manometrically. Elitsur[106] employed anhydrous ethanol as a reaction medium although Perkin and Pratt[109] had demonstrated earlier that calcium hydride reacted with alcohols, forming the calcium alkoxide and hydrogen. This observation was verified by Perryman,[108] who recommended that anhydrous dioxane be used in place of alcohol as a water extractant, and by Fischer,[110] who found that active compounds such as methanol and acetone interfered to a considerable extent. The method, although time consuming, was capable of quite high precision.

In a limited number of cases the Grignard technique has been employed. Larson[111] used methyl magnesium iodide for the determination of moisture in oil:

$$CH_3MgI + H_2O \longrightarrow CH_4 + MgI(OH) \qquad (5)$$

measuring the methane released. This method was confined to materials in which no other compounds containing active hydrogen were present.

The most widely applicable procedures for the determination of water in organic materials have been based on titrimetry. Magnesium nitride combined with water releasing ammonia which could be absorbed and titrated with standard sulfuric acid solution:[112–114]

$$Mg_3N_2 + 6\,H_2O \longrightarrow 3\,Mg(OH)_2 + 2\,NH_3 \qquad (6)$$

Sodamide could be employed in the same way:[114]

$$NaNH_2 + H_2O \longrightarrow NaOH + NH_3 \qquad (7)$$

α-Naphthoxydichlorophosphine,[115,116] its oxide,[117] and α-naphthyl-

---

[104] Rosenbaum, C. K., and Walton, J. H., *J. Am. Chem. Soc.*, 52, 3568–73 (1930).

[105] Notevarp, O., *Z. anal. Chem.*, 80, 21–56 (1930).

[106] Elitsur, A. G., *J. Gen. Chem. U.S.S.R.*, 10, 1981–4 (1940); 14, 924–34 (1944). Also, *J. Applied Chem. U.S.S.R.*, 14, 682–8 (1941).

[107] Broche, H., and Scheer, W., *Brennstoff-Chem.*, 13, 281–5 (1932).

[108] Perryman, P. W., *Analyst*, 70, 45–7 (1945).

[109] Perkin, F. M., and Pratt, L., *J. Chem. Soc.*, 95, 159–65 (1909).

[110] Fischer, K., *Angew. Chem.*, 48, 394–6 (1935).

[111] Larson, R. G., *Ind. Eng. Chem., Anal. Ed.*, 10, 195–8 (1938).

[112] Dietrich, K. R., and Conrad, C., *Angew. Chem.*, 44, 532–4 (1931).

[113] Shatalov, V. P., *Sintet. Kauchuk*, 5, 31–3 (1936).

[114] Roth, F., and Schultz, A., *Brennstoff-Chem.*, 20, 317 (1939).

[115] Bell, R. P., *J. Chem. Soc.*, 1932, 2903–5.

[116] Lindner, J., and Zienert, G., *Mikrochemie ver. Mikrochim. Acta*, 31, 254–62 (1943).

[117] Kunz, P., *Ber.*, 27, 2559–65 (1894).

dichlorophosphine oxide,[118] in the presence of water, all quantitatively released hydrogen chloride which could be titrated.

$$C_{10}H_7{\cdot}O{\cdot}PCl_2 + 2\,H_2O \longrightarrow C_{10}H_7{\cdot}O{\cdot}P(OH)_2 + 2\,HCl \qquad (8)$$

$$C_{10}H_7{\cdot}O{\cdot}POCl_2 + 2\,H_2O \longrightarrow C_{10}H_7O{\cdot}PO(OH)_2 + 2\,HCl \qquad (9)$$

$$C_{10}H_7{\cdot}POCl_2 + 2\,H_2O \longrightarrow C_{10}H_7{\cdot}O{\cdot}P(OH)_2 + 2\,HCl \qquad (10)$$

Sodium triphenylmethyl, employed by Bent and Lesnick[119] to measure the amount of water absorbed on glass surfaces, was found applicable to the determination of water in benzene,[120] the reagent serving as its own indicator. The reagent required a rather complex apparatus and was subject to interference from a wide variety of active materials. Henle[121] has described the use of aluminum ethoxide for the estimation of water in organic compounds:

$$Al(OC_2H_5)_3 + 3\,H_2O \longrightarrow Al(OH)_3 + 3\,C_2H_5OH \qquad (11)$$

in which the precipitated aluminum hydroxide was recovered, dehydrated, and weighed as the oxide.

Smith and his coworkers[122,123] recommended their sodium–ester method for the determination of small quantities of water in alcohol:

$$Na + H_2O \longrightarrow NaOH + \tfrac{1}{2}\,H_2 \qquad (12)$$

$$Na + ROH \longrightarrow RONa + \tfrac{1}{2}\,H_2 \qquad (13)$$

$$NaOH + CH_3COOC_2H_5 \longrightarrow CH_3COONa + C_2H_5OH \qquad (14)$$

A small piece of sodium (an excess over the water present) was added to a known volume of sample. Since the rate of reaction between sodium and water was considerably greater than that between the metal and alcohol, reaction (12) went to completion while the excess sodium formed the alkoxide (equation 13). Total base was determined either by the use of a known weight of sodium or, preferably, by acidimetric titration of a portion of the solution. After reaction of another aliquot with ethyl acetate, during which time the sodium hydroxide was converted to sodium salt (equation 14), the alkoxide was determined specifically by titration. This latter titer actually was a measure of excess sodium and the net value,

---

[118] Lindner, J., *Z. anal. Chem.*, 66, 305–70 (1925).

[119] Bent, H. E., and Lesnick, G. S., *J. Am. Soc.*, 57, 1246–50 (1935).

[120] Simons, J. H., and Kipp, E. M., *Ind. Eng. Chem., Anal. Ed.*, 13, 328–30 (1941).

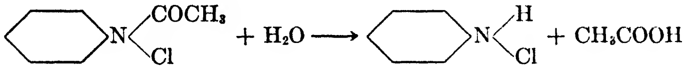
[121] Henle, F., *Ber.*, 53, 719–22 (1920).

[122] Smith, E. L., *J. Chem. Soc.*, 1927, 1284–8.

[123] Gyngell, E. S., Phillips, M. A., and Smith, E. L., *Ind. Chemist*, 21, 526–32 (1945).

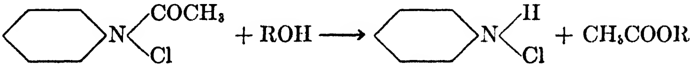
therefore, represented the sodium hydroxide which was equivalent to the total water originally present. Although time consuming and indirect, the method was capable of quite high precision and accuracy.

The ease with which some anhydrides and acyl chlorides are hydrolyzed has led to the development of several quantitative procedures employing these reactions. Among the most useful was that described by Smith and Bryant[124] based on the room temperature hydrolysis of acetyl chloride in the presence of pyridine, producing 2 moles of titrable acid:



(15)

The acetylpyridinium chloride, on the other hand, produced only 1 mole of acid on reaction with dry alcohol:



(16)

In practice the sample to be analyzed for water content was added to an excess of reagent (acetyl chloride in purified dioxane or toluene) stabilized with pyridine. After a few minutes at room temperature the unused acetyl chloride was decomposed by absolute ethanol. The increase in acidity of the sample over the blank, determined by titration with standard alkali, was a direct measure of the water present in the sample, 1 mole of water liberating 1 extra mole of acid. The method was about 97.5 per cent stoichiometric and was capable of detecting as little as 0.02 per cent of water. It was subject to interference from formic acid, aldehydes, and high concentrations of the lower alcohols and glycols. Strong amines, aldehydes, and easily hydrolyzed esters interfered by making the acidimetric endpoint rather indefinite. Fatty acids increased the titration proportionately; it was necessary to determine them by an independent method in order that the necessary stoichiometric correction could be applied. Kaufmann and Funke[125] modified the Smith-Bryant method in applying it to the analysis for moisture in fats, butter, and margarine by reducing the concentration of acetyl chloride and employing carbon tetrachloride as solvent. In

---

[124] Smith, D. M., and Bryant, W. M. D., *J. Am. Chem. Soc.*, 57, 841–5 (1935).
[125] Kaufmann, H. P., and Funke, S., *Fette u. Seifen*, 44, 386–90 (1937).

some cases aniline instead of alcohol was used to decompose the excess reagent.

Later Finken and Hölters[126] showed that the acetyl chloride procedure could be used for the determination of water in sulfonated oils and Levin and his coworkers[127] demonstrated successfully that this technique might be employed for the determination of as little as 0.001 per cent moisture in hydrocarbon gases. The gas to be analyzed was contacted with dry acetone and pyridine at about −60°C. where the moisture was extracted. Subsequent analysis of the acetone solution gave an accurate measure of the original moisture concentration of the gas. Cinnamoyl chloride was recommended as being a suitable reagent for the determination of water[128] but was not as generally applicable as acetyl chloride, since it was not as easily hydrolyzed. However, it might be useful as a liquid absorbent for moisture if used above its melting point of 36°C. (probably in the range 60–65°C.).

The rate of hydrolysis of acid anhydrides in the presence of acid or basic catalysts was considerably slower than that of acetyl chloride; methods based on the use of anhydrides were more time consuming and subject to more types of interference. One of the earliest reported procedures was based on the use of benzoic anhydride:[129]

$$(C_6H_5CO)_2O + H_2O \longrightarrow 2\ C_6H_5COOH \qquad (17)$$

The sample was allowed to react from four to twelve hours at 110°C. On cooling in an ice bath the total benzoic acid was titrated with standard alkali. (The low solubility and negligible hydrolysis of benzoic anhydride in cold water[130] made possible the selective titration of benzoic acid in the presence of the anhydride at 0–10°C.) Alcohols also reacted:

$$(C_6H_5CO)_2O + ROH \longrightarrow C_6H_5COOH + C_6H_5COOR \qquad (18)$$

Ross[129] corrected for alcohol interference by hydrolyzing the excess anhydride, after the first acidimetric titration was completed. Knowing the moles of anhydride used and the moles of benzoic acid

---

[126] Finken, H., and Hölters, H., *Ibid.*, 46, 70 (1939).

[127] Levin, H., Uhrig, K., and Roberts, F. M., *Ind. Eng. Chem., Anal. Ed.*, 17, 212–15 (1945).

[128] van Nieuwenburg, C. J., *Chem. Weekblad*, 34, 217 (1937).

[129] Ross, J., *J. Soc. Chem. Ind.*, 51, 121–2 (1932).

[130] Wilsdon, B. H., and Sidgwick, N. V., *J. Chem. Soc.*, 103, 1959–73 (1913).

formed, he was able to calculate the alcohol concentration and thus correct the initial titer. Formaldehyde, but not acetaldehyde, interfered with the method through the formation of methylene dibenzoate:

$$(C_6H_5CO)_2O + HCHO \longrightarrow (C_6H_5COO)_2CH_2 \qquad (19)$$

An approximate correction was applied after isolation and gravimetric determination of the ester thus formed, permitting a calculation of the benzoic anhydride used up in this way. Acetal, but not methylal, interfered to some extent:

$$CH_3CH(OC_2H_5)_2 + (C_6H_5CO)_2O \longrightarrow CH_3CHO + 2\ C_6H_5COOC_2H_5 \qquad (20)$$

The lower fatty acids interfered through interaction with benzoic anhydride to form the corresponding lower anhydride as well as the mixed anhydride. These hydrolyzed with greater ease and did not permit the specific titration of free acid alone as called for by the analysis. Simple ketones and hydrocarbons were found to be inactive toward the anhydride.

Toennies and Elliott[131] described a technique employing acetic anhydride dissolved in acetonitrile or ether, less drastic but more time consuming than that of Ross. The hydrolysis was accelerated by the use of catalytic quantities of 2,4-dinitrobenzenesulfonic acid (comparable in acid strength to the strong mineral acids). After the reagent and sample, for which the water content was desired, had been allowed to stand several hours at room temperature, the increase in acidity of sample over blank was determined by titration with sodium methylate using thymol blue indicator:

$$(CH_3CO)_2O + H_2O \longrightarrow 2\ CH_3COOH \qquad (21)$$

$$CH_3COOH + NaOCH_3 \rightleftharpoons CH_3COONa + CH_3OH \qquad (22)$$

$$(CH_3CO)_2O + NaOCH_3 \longrightarrow CH_3COONa + CH_3COOCH_3 \qquad (23)$$

Since, after hydrolysis, 2 moles of titrable acid were formed while the anhydride alone used only 1 equivalent of sodium methylate, the increase in acidity was a direct measure of original water content of the sample. Less than 0.001 per cent water was determined by this method. However, it was subject to interference from formic acid, acetone, and other active compounds.

If alcohol was to be determined the excess anhydride was destroyed with added water in which case the decrease in acidity of the sample over blank be-

---

[131] Toennies, G., and Elliott, M., *J. Am. Chem. Soc.*, 59, 2136–9 (1937).

came a measure of alcoholic hydroxyl. This technique was analogous to the acetyl chloride procedures of Smith and Bryant.[124,132] If desirable, other anhydrides or acyl chlorides could be employed in a similar manner.

In 1935 a water method of unusual simplicity was described by Karl Fischer.[110] His reagent, a solution of iodine, sulfur dioxide, and pyridine in methanol, has been the basis of the most widely applicable, and one of the most rapid, titrimetric procedures available for the determination of any quantity of water in organic and inorganic materials.

[132] Smith, D. M., and Bryant, W. M. D., *Ibid.*, 57, 61–5 (1935).

CHAPTER II

Titrimetric Determination of Water Using Karl Fischer Reagent

Summary of Analytical Procedures Based on Absorption or Elimination of Water*

A. Reagent Specifications

The qualities of all the compounds used in the preparation of Karl Fischer reagent and in its applications are summarized in Table I.

B. Preparation of Karl Fischer Reagent

Substance	Quantity per liter of reagent	Total quantity
Pyridine	269 ml.	2420 ml.
Iodine	84.7 grams	762 grams
Methanol	667 ml.	6000 ml.
Sulfur dioxide	45 ml. (64 grams)	135 ml.

The stock reagent is prepared by dissolving 762 grams of iodine in 2420 ml. of pyridine in a 9 liter glass-stoppered bottle. Then 6 liters of methanol are added. In preparing the active reagent 3 liter quantities of the stock solution are transferred to 4 liter buret bottles and cooled by placing the bottles in a slurry of chopped ice. About 135 ml. of liquid sulfur dioxide, collected in a calibrated cold trap, are added carefully and the bottle stoppered. The mixture is shaken until homogeneous and set aside for a day or two before use.

C. Preparation of Standard Water-in-Methanol Solution

About 15 grams of water (accurately weighed) are diluted to exactly 1 liter with methanol at 25 ± 1°C. The solution is trans-

---

* This chapter is intended as a ready reference. It should be used only by those thoroughly familiar with the more detailed procedures given in later chapters, particularly as regards scope, safety and other precautions, and interferences for each method.

TABLE I

Specifications for Compounds Used in Karl Fischer Techniques

Substance	Grade	Purification or preparation reference (Chapter No.)	Water tolerance, wt. %	Reliable sources[a]
Acetic acid	C.P., glacial		0.2	Merck & Co.
Acetic anhydride	C.P. (<0.5% acid as CH_3COOH)		0.0	Eastman Kodak Co.
Benzaldehyde	Pure (<0.2% as C_6H_5COOH)	XIII	0.1	Eastman Kodak Co.
Boron trifluoride	97%		0.1	The Matheson Co., Ohio Chemical Co.
Boron trifluoride–acetic acid	$BF_3 \cdot 2CH_3COOH$	IX	0.2	
Boron trifluoride–etherate	$BF_3 \cdot C_2H_5OC_2H_5$, technical		0.3	Eastman Kodak Co., General Chemical Co.
Boron trifluoride–methanol	$BF_3 \cdot 2CH_3OH$	X	0.2	
n-Butanol	C.P.		1.0	Eastman Kodak Co.
Dichloroacetic acid	C.P.	IX	0.2	Eastman Kodak Co., Eimer & Amend
Diethylene glycol	Technical		1.0	Carbide & Carbon Chemical Co., Eastman Kodak Co.
Dioxane	Technical		0.3	Carbide & Carbon Chemical Co.
Dioxane	Purified	XIV	0.0	
Hydrogen cyanide	Technical		3.5	American Cyanamid Co.
Hydroxylamine hydrochloride	C.P. (<0.5% acid as HCl)		0.5	Eastman Kodak Co., Eimer & Amend

Iodine	U.S.P., resublimed		0.0	Mallinckrodt Chem. Works, Merck & Co., J. T. Baker Chem. Co.
Isooctene	Practical		0.0	Eastman Kodak Co., Phillips Petroleum Co.
Methanol	C.P.		0.1	E. I. du Pont de Nemours & Co., Carbide & Carbon Chemical Co.
Potassium hydroxide	C.P. pellets		2.5	Mallinckrodt Chemical Works
Pyridine	C.P.	IX	0.1	J. T. Baker Chem. Co., Eastman Kodak Co., Barrett Chem. Co.
Sodium cyanide	C.P.	V	0.0	J. T. Baker Chem. Co., Merck & Co.
Sodium hydroxide	C.P. pellets		2.5	Mallinckrodt Chemical Works, Merck & Co., J. T. Baker Chem. Co.
Sodium iodide	C.P.		0.5	Merck & Co., J. T. Baker Chem. Co.
Sulfur dioxide	99.5%		0.5	Virginia Smelting Co., The Matheson Co.
Zinc	C.P., dust, 90% min.		0.0	Mallinckrodt Chem. Works, Merck & Co.

---

[a] Doubtless chemicals meeting the above specifications are available from other sources. Within the experience of the authors those companies listed have supplied chemicals of the desired purity.

ferred to a dry glass-stoppered 1 liter Pyrex bottle and stored in a thermostat at 25 ± 1°C.

Total water concentration of a 10 ml. portion of the water standard is calculated from the quantity of moisture in 9.85 ml. of methanol plus 1 per cent of the water added.

D. Standardization of the Reagent

Ten milliliters of the standard water in methanol solution are transferred to a 250 ml. glass-stoppered volumetric flask and titrated with the Fischer reagent.

Calculation:

Milligrams of water added divided by milliliters of Fischer reagent required equals milligrams of water per milliliter of Fischer reagent.

E. Determination of Free Water or Water of Hydration in Inert Compounds (General Procedure)

Most substances are inert to Karl Fischer reagent. A number of compounds and classes of compounds, however, react stoichiometrically with one or more of the components of Fischer reagent. Included among these are (unless otherwise indicated these substances are discussed in Chapter VIII):

- *l*-Ascorbic acid (Chapter V)
- Hydrazine salts (Chapter V)
- Substituted hydrazine salts (Chapter V)
- Mercaptans (Chapter XV)
- Alkali bicarbonates
- Alkali carbonates
- Alkali sulfites
- Alkali pyrosulfites
- Boric acid and oxides
- Cupric salts
- Ferric salts
- Metal hydroxides
- Metal oxides
- Sodium arsenate
- Sodium arsenite
- Sodium tetraborate
- Sodium thiosulfate
- Stannous chloride

In general these reactive compounds either may be rendered inert (excess acetic acid eliminates amine and hydrazine interference) or may be estimated by an independent method and a stoichiometric correction applied to the water determination.

1. IN SOLUBLE MATERIALS

The sample, containing up to 250 milligrams of water (preferably 50 to 200 milligrams), is weighed into a 250-ml. glass-stoppered volumetric flask. Twenty-five milliliters of methanol are added and the mixture titrated directly with Karl Fischer reagent. The water content of the methanol is determined by titration of an equal volume in the same type of flask.

Calculation:

$$\frac{(\text{Sample ml.} - \text{Blank ml.}) \times \text{mg. water per ml. K.F.}}{1000 \times \text{weight sample (grams)}} \times 100 = \text{per cent water}$$

2. In Porous Solids

If finely divided, the sample is dispersed in 50 to 100 ml. of dry methanol, allowed to stand with occasional shaking for periods up to 1 hour at room temperature, and titrated with Karl Fischer reagent.

Solids requiring more rigorous extraction are weighed into a special 500 ml. flask ("Hydractor") or into a Waring Blendor flask. Then 100 ml. of dry methanol are added and the mixture agitated for 10 to 15 minutes at room temperature (see Chapter VI for description of apparatus). At the end of this time, the mixture or an aliquot is titrated with Fischer reagent.

F. Determination of Free Water in Compounds That Interfere on Direct Titration

1. In Carbonyl Compounds

Relatively stable aldehydes and ketones are handled like inert compounds except that they are diluted with 25 ml. of pyridine instead of methanol and titrated to the first appearance of iodine.

Active carbonyl compounds react to varying extents with Fischer reagent and must be combined as the cyanohydrins before titrating for water. (This procedure is carried out in a well-ventilated hood).

Reagents		Concentration
Active ingredient	Solvent (ml.)	
(1) Hydrogen cyanide (70 ml.)	Dioxane (pure) (930)	5%
(2) Sodium cyanide (1 gram)	Methanol (100)	1%
(3) Pyridine		C.P.

Ten milliliters of sodium cyanide solution and 20 ml. of hydrogen cyanide reagent are added to a 250 ml. glass-stoppered volumetric flask. The sample, containing no more than 30 millimoles of carbonyl, is added, followed by 20 ml. of pyridine. The mixture and a corresponding blank are shaken and allowed to stand 30 minutes at room temperature, after which they are titrated with Karl Fischer reagent.

2. IN MERCAPTANS

All mercaptans react stoichiometrically with Fischer reagent and must be combined as sulfides before titrating for water.

Reagents		Concentration
Active ingredient (ml.)	Solvent (ml.)	
(1a) $BF_3 \cdot 2CH_3COOH$ (210) or	Acetic acid (790)	100 g./liter
(1b) $BF_3 \cdot (C_2H_5)_2O$		Technical
(2) Isooctene		Technical
(3) Acetic acid		C.P. glacial
(4) Pyridine		C.P.

Five milliliters of acetic acid are added to a 250 ml. glass-stoppered volumetric flask. The sample, containing up to 15 millimoles of mercaptan, is added, followed by 10 ml. of boron fluoride in acetic acid (or 3 ml. of boron fluoride–ether) and 6 ml. of isooctene. The mixture and the corresponding blank are shaken and allowed to stand for 30 minutes at room temperature. Then 5 ml. of pyridine are added carefully to each flask and the solutions titrated with Karl Fischer reagent.

G. Analytical Procedures Employing Karl Fischer Reagent

1. GENERAL

The several analytical procedures employing Karl Fischer reagent are summarized in Table II. All of these procedures are based on quantitative reactions in which water is either eliminated or absorbed. From an examination of Table II it is evident that the analytical procedures have many points in common. For example, a solution of boron trifluoride in acetic acid is used as a reagent for the quantitative determination of acetals, alcohols, amino alcohols, anhydrides, nitriles, ortho esters, and dialkyl peroxides. In the esterification procedures, respectively, for alcohols and acids the strictly aromatic compounds react only partially. The other condensation reactions, in general, are applicable to all types of compounds, although the effects of steric hindrance frequently are evident, as, for example, in the difficult acetylation of diaryl secondary amines and in the limited reactions of camphor and camphoric acid.

Interfering reactions in all of the procedures usually are quantitative or nearly so (90 per cent or more). Therefore, where independent methods of analysis for these compounds are available, a suitable correction frequently may be applied particularly for routine samples of approximately constant composition. In the procedure for primary amines, based on the imine reaction, ordinary secondary amines do not interfere but samples containing alkamines with a secondary amino nitrogen group undergo a nearly quantitative cyclization reaction in which water is eliminated. In the determination of nitriles in the presence of alcohols, compensating reactions occur and, consequently, where both groups are present in equivalent amounts, the net reaction is zero.

Materials which are easily oxidized or reduced or which enter readily into reactions involving water may interfere in a wide variety of procedures in which, however, they may be encountered rarely; among organic compounds, aldehydes and peroxides are outstanding in these respects but the interference usually can be eliminated or minimized by appropriate procedural variations. Often by a slight modification of the method an otherwise interfering reaction can be made to go to completion. A short heating period, for example, is sufficient to effect quantitative reaction of primary hydroxyl in the acetylation procedure for primary plus secondary amines which react at room temperature.

In general a weighed sample is diluted to a fixed volume, thermostating at 25 ± 1°C. if a time interval is involved between removals of aliquots. Portions of this solution, containing up to 10 milliequivalents of the active functional group, are transferred with precision (calibrated) pipets to 250 ml. glass-stoppered volumetric flasks. In all cases one or more blanks are run, substantially identical with the sample runs in all respects except for omission of the samples. (Where desirable, of course, the sample may be weighed directly into the 250 ml. volumetric flask. In this case the solvent is eliminated from the blank.)

Exact corrections are determined by appropriate titrations for all free water originally present in the sample, diluents, and reagents. In calculating the moisture content of the sample in the aliquot it is assumed that there is no volume change on mixing. (The error in this assumption is usually well within the experimental uncertainty.) The correction for water in the solvent is figured from the difference between the total volume of the aliquot

TABLE II

Analytical Procedures Employing Karl Fischer Reagent

No.	Analysis for main reaction	Principal reagents	Application	Interfering compounds, incomplete reactions	Chapter references
1	Acetals $R(OR')_2 + 4\ CH_3COOH = 2\ CH_3COOR' + (CH_3COO)_2R + 2\ H_2O$	100 g. BF_3/l. CH_3COOH solution	Straight chain acetals (80-90%)	See alcohols (No. 3)	IX
2	Acyl halides $RCOX + H_2O = RCOOH + HX$	H_2O, C_5H_5N	Acetyl chloride, acetylsulfanilyl chloride	Anhydrides	XV
3	Alcoholic hydroxyl $ROH + CH_3COOH = CH_3COOR + H_2O$	100 g. BF_3/l. CH_3COOH solution	Primary, secondary, and tertiary aliphatic and alicyclic alcohols	Aldehydes, cyclohexanone, acetals, nitriles, mercaptans, ortho esters, phenols	IX
4	Alcohols	25 g. BF_3/l. CH_3COOH solution	Aliphatic alcohols in presence of phenols	See alcohols (No. 3)	IX
5	Alcohols	$CHCl_2COOH$	Aliphatic in presence of phenols	Aldehydes mercaptans, ortho esters	IX
6	Amino alcohols	200 g. BF_3/l. CH_3COOH solution	Primary, secondary, and tertiary aliphatic and alicyclic amino alcohols	See alcohols (No. 3)	IX
7	Amides $RCONH_2 + KOH = RCOOK + NH_3$	1 *N* KOH in $HOCH_2CH_2OCH_2CH_2OH$	Formamide, acetamide, adipamide	Esters, acids	XIII
8	Amines $RR'NH + (CH_3CO)_2O = RR'NOCCH_3 + CH_3COOH$	1.5 *M* $(CH_3CO)_2O$ in C_5H_5N, 10% NaI + 2.2% H_2O in C_5H_5N	Primary and secondary amines	*Sec* and *tert* alcohols, pyrrole, diaryl secondary amines	XIII

9	Amines $RNH_2 + C_6H_5CHO = C_6H_5CH{=}NR + H_2O$	C_6H_5CHO, 6% HCN in C_5H_5N	Primary amines	Heterocyclic secondary amines, amino alcohols having secondary amino-nitrogen, urea	XIII
10	Anhydrides $(RCO)_2O + H_2O = 2\ RCOOH$	100 g. BF_3 + 6.5 ml. H_2O/l. CH_3COOH solution	Anhydrides of carboxylic acids	See alcohols (No. 3), maleic, phthalic, and succinic anhydrides	XI
11	Anhydrides	10% NaI + 1% H_2O in C_5H_5N	Anhydrides of carboxylic acids	Maleic, camphoric anhydrides	XI
12	Carbonyl compounds $RCHO + H_2NOH{\cdot}HCl = RCH{=}NOH + H_2O + HCl$	0.5 *N* $NH_2OH{\cdot}HCl$ in CH_3OH, 1 *M* $C_5H_5N{\cdot}SO_2$ in CH_3OH	Aliphatic, alicyclic, and aromatic aldehydes and ketones	Peroxides, anhydrides, camphor	XII
13	Carboxylic acids $RCOOH + CH_3OH = RCOOCH_3 + H_2O$	100 g. BF/l. CH_3OH solution	Aliphatic and alicylic acids	Aldehydes, anhydrides, ketones, sulfuric acid, amino acids, furoic acid, camphoric acid, aromatic acids	X
14	Carboxylic acids	25 g. BF_3/l. CH_3OH solution	Aliphatic in presence of aromatic acids	See acids (No. 13)	X
15	**Esters**	1 *N* NaOH + 1% H_2O in CH_3OH	Simple acetates and propionates	Acids, aldehydes, amides	XV

Table (*continued*)

TABLE II (*concluded*)

No.	Analysis for main reaction	Principal reagents	Application	Interfering compounds, incomplete reactions	Chapter references
16	Esters $RCOOR' + KOH = RCOOK + R'OH$	1 *N* KOH in $HOCH_2CH_2$-OCH_2CH_2OH 1 *N* NaOH + 1% H_2O in C_4H_9OH	Higher esters	Acids, aldehydes, amides	XV
17	Metal salts $RCOOM + CH_3OH = RCOOCH_3 + MOH$ $MOH + I_2 + SO_2 + CH_3OH = MI + HI + HSO_4CH_3$	100 g. BF_3/l. CH_3OH solution	Metal salts of carboxylic acids	See acids (No. 13)	XV
18	Nitriles $RCN + H_2O = RCONH_2$	6.5 ml. H_2O/500 ml. $BF_3 \cdot 2CH_3COOH$ solution	Aliphatic and aromatic nitriles	See alcohols (No. 3)	XIII
19	Ortho esters $RC(OR')_3 + 2\ CH_3COOH = RCOOR' + 2\ CH_3COOR' + H_2O$	100 g. BF_3/l. CH_3COOH solution	Ortho esters of aliphatic acids	See alcohols (No. 3)	IX
20	Peroxides $(RCOO)_2 + 2\ HI = 2\ RCOOH + I_2$	"Discharged" Karl Fischer reagent	Diacyl peroxides		XIV
21	Peroxides $ROOR + H_2 = 2\ ROH$ $ROOH + H_2 = ROH + H_2O$ $ROH + CH_3COOH = CH_3COOR + H_2O$	Zn, 100 g. BF_3/l. CH_3-$COOH$ solution	Dialkyl peroxides	See alcohols (No. 3)	XIV

and the volume of concentrated sample which it contains. These points are illustrated in the following sample calculations:

(*a*) *Calculation for an analysis of a sample containing an alcohol (applicable to the procedures for acids, primary amines, carbonyl compounds, dialkyl peroxides).* A 10.3765 gram sample of allyl alcohol (M.W. = 58.10, d = 0.85) was diluted to exactly 100 ml. with technical dioxane. A 5 ml. aliquot contained 0.5188 gram (0.61 ml.) of sample and about 4.4 ml. of dioxane.

Solution	Ml. Fischer reagent (0.1629 millimole H_2O/ml.)	Water found, millimoles	
		Gross	Net
5 ml. dioxane	2.6	(a) 0.42	(a') 0.37/4.4 ml.
5 ml. aliquot (free water analysis)	8.3	(b) 1.35	(b') 0.98 $(b - a')$ = 3.4% H_2O
5 ml. dioxane + 20 ml. catalyst + 5 ml. pyridine (blank)	27.2	(c) 4.43	(c') 4.38 $(c - a + a')$
5 ml. aliquot + 20 ml. catalyst + 5 ml. pyridine (sample analysis)	85.3	(d) 13.90	(d') 8.54 $(d - c' - b')$

$$\frac{8.54 \times 58.10}{1000 \times 0.5188} \times 100 = 95.6\% \text{ allyl alcohol}$$

$$95.6\% + 3.4\% = 99.0\% \text{ total alcohol plus water}$$

(*b*) *Calculation for an analysis of a sample containing a nitrile (applicable to the procedures for anhydrides and acyl chlorides).* A 2.2481 gram sample of *n*-valeronitrile (M.W. = 83.18, d = 0.95) was diluted to exactly 25 ml. with acetic acid. Five milliliter portions contained 0.4496 gram (0.5 ml.) of sample and about 4.5 ml. of acetic acid.

Solution	Ml. Fischer reagent (0.1959 millimole H_2O/ml.)	Water found, millimoles	
		Gross	Net
5 ml. acetic acid	5.3	(a) 1.04	(a') 0.94/4.5 ml.
5 ml. aliquot (free water analysis)	5.4	(b) 1.06	(b') 0.12 $(b - a')$ = 0.5% H_2O
5 ml. acetic acid + 20 ml. catalyst + 10 ml. pyridine (blank)	89.6	(c) 17.55	(c') 17.45 $(c - a + a')$
5 ml. aliquot + 20 ml. catalyst + 10 ml. pyridine (sample analysis)	62.3	(d) 12.20	(d') 5.37 $(c' + b' - d)$

$$\frac{5.37 \times 83.18}{1000 \times 0.4496} \times 100 = 99.4\% \text{ valeronitrile}$$

$$99.4\% + 0.5\% = 99.9\% \text{ total nitrile plus water}$$

(*c*) *Calculation for an analysis by acetylation of a sample containing primary amine.*

A 4.6899 gram sample of 2-aminopyridine (M.W. = 94.16, d = 1.00) was diluted to exactly 50 ml. with pyridine. Ten milliliter portions contained 0.9374 gram (0.94 ml.) of sample and about 9.1 ml. of pyridine.

Solution	Ml. Fischer reagent (0.1485 millimole H_2O/ml.)	Water found, millimoles	
		Gross	Net
10 ml. CH_3COOH	8.4	(*a*) 1.25	
10 ml. C_5H_5N	4.5	(*b*) 0.67	(*b*′) 0.61/9.1 ml.
10 ml. aliquot + 10 ml. CH_3COOH (free water analysis)	12.9	(*c*) 1.92	(*c*′) 0.06 ($c - a - b'$) = 0.1% H_2O
20 ml. $(CH_3CO)_2O$ reagent + 10 ml. C_5H_5N + 25 ml. NaI reagent (control) (25 ml. pipet calibration = 0.9990)	61.7	(*d*) 9.16/24.98 ml.	
10 ml. NaI solution (hydrolysis reagent) (10 ml. pipet cal. = 0.9965)	100.8	(*e*) 14.95/9.965 ml.	
Calculated H_2O/24.98 ml.		(*f*) 37.51 (from *e*)	
10 ml. aliquot + reagents (see *d* above) after reaction (sample analysis)	128.5	(*g*) 19.09	

Acetic anhydride added:

from (*f*)	37.51	millimoles	H_2O in NaI reagent
(*b*)	0.67	"	H_2O in 10 ml. C_5H_5N
	38.18	"	total H_2O added
from (*d*)	9.16	"	H_2O
	29.02	"	H_2O ≎ $(CH_3CO)_2O$

Sample added:

from (*f*)	37.51	millimoles	H_2O in NaI reagent
($b' + c'$)	0.67	“	in 9.1 ml. C_5H_5N + 0.9 ml. amine
	38.18	“	total H_2O added
from (*g*)	19.09	“	H_2O found
	19.09	“	H_2O used up $\asymp$ $(CH_3CO)_2O$ not acetylated

29.02 − 19.09 = 9.93 millimoles $(CH_3CO)_2O$ used up $\asymp$ amine present

$$\frac{9.93 \times 94.16}{1000 \times 0.9374} \times 100 = 99.7\% \text{ 2-aminopyridine}$$

$$99.7\% + 0.1\% = 99.8\% \text{ total amine plus water}$$

One of the three sample calculations will be applicable to any of the analytical procedures employing Karl Fischer reagent. The example (*a*) for alcohols is used for all reactions in which water is eliminated and (*b*) for nitriles, for analyses in which water is absorbed. The calculation (*c*) for primary plus secondary amines represents the case where water used up becomes a measure of unreacted reagent (anhydride).

The procedures to follow present in summary form the principal applications, up to the present time, of the Karl Fischer reagent in the determination of organic functional groups.

2. DETERMINATION OF ALCOHOLIC HYDROXYL

$$ROH + CH_3COOH \xrightarrow{BF_3} CH_3COOR + H_2O \qquad (1)$$

This general technique is applicable also to the determination of acetals and ortho esters.

$$R(OR')_2 + 4\ CH_3COOH \longrightarrow 2\ CH_3COOR' + (CH_3COO)_2R + 2H_2O \quad (1a)$$

$$RC(OR')_3 + 2\ CH_3COOH \longrightarrow RCOOR' + 2\ CH_3COOR' + H_2O \quad (1b)$$

Reagents. (1) Esterification catalyst solution: 210 ml. of $BF_3 \cdot 2\ CH_3COOH$ diluted to 1 liter with acetic acid $\asymp$ 100 grams of BF_3 per liter.

(2) Dioxane, technical, or acetic acid.

(3) Pyridine.

Procedure. A 5 ml. aliquot from a dioxane or acetic acid solution of the sample, containing up to 10 milliequivalents of hydroxyl (but not more than 15 milliequivalents of hydroxyl plus water), plus 20 ml. of the esterification catalyst solution is heated 2 hours at 67 ± 2°C. After cooling to room temperature spontaneously, 5 ml. of pyridine are added carefully and the solution is titrated with Karl Fischer reagent.

At least one blank, containing 5 ml. of solvent, 20 ml. of catalyst solution, and 5 ml. of pyridine is run with each set of samples.

3. Determination of Amino Alcohols

$$H_2NROH + CH_3COOH \xrightarrow{BF_3} H_2NROOCCH_3 + H_2O \qquad (2)$$

Reagents. (1) Esterification catalyst solution: 420 ml. of $BF_3 \cdot 2CH_3COOH$ diluted to 1 liter with acetic acid $\Bumpeq$ 200 grams of BF_3 per liter.

(2) Acetic acid.

(3) Pyridine.

Procedure. Essentially the same as that for alcohols described above. In this case 10 ml. of pyridine (instead of 5 ml.) are added carefully just before titration with Fischer reagent.

4. Determination of Primary Plus Secondary Amines

$$RR'NH + (CH_3CO)_2O \longrightarrow RR'NOCCH_3 + CH_3COOH \qquad (3)$$

$$(CH_3CO)_2 + H_2O \longrightarrow 2\ CH_2COOH \qquad (4)$$

Reagents. (1) Acetylating reagent: 142 ml. of $(CH_3CO)_2O$ diluted to 1 liter with pyridine $\Bumpeq$ 1.5 molar.

(2) Hydrolysis reagent: 100 grams of NaI plus 22.0 ml. of water (total) in 1 liter of pyridine solution.

(3) Pyridine.

(4) Acetic acid.

Procedure. A 5 or 10 ml. aliquot from a pyridine solution of the sample, containing up to 10 milliequivalents of primary plus secondary amine, and 20 ml. of acetylating reagent are allowed to stand 30 minutes at room temperature. Then exactly 25 ml. (calibrated pipet) of hydrolysis reagent are added and the mixture heated 30 minutes at $60 \pm .1$°C. After cooling to room temperature spontaneously, the solution is titrated with Karl Fischer reagent.

Twenty milliliters of the acetylating reagent are standardized by standing with 5 or 10 ml. of the pyridine used as solvent for 30 minutes at room temperature and then treating with 25 ml. (calibrated pipet) of hydrolysis reagent, as above. The water content of the hydrolysis reagent is calculated from the titer of 10 ml. (calibrated pipet) portions with Karl Fischer reagent.

Acetic acid is used in general to combine with the sample for the determination of free water. This is unnecessary for amines weaker than pyridine.

5. Determination of Primary Amines

$$RNH_2 + C_6H_5CHO \longrightarrow C_6H_5CH = NR + H_2O \qquad (5)$$

Reagents. (1) C_6H_5CHO, acid free.

(2) HCN solution: 42 ml. of liquid HCN diluted to 500 ml. with pyridine ≈ 6 per cent HCN.

(3) Sodium cyanide, anhydrous.

(4) Pyridine.

(5) Acetic acid.

Procedure. A 5 ml. portion from a pyridine solution of the sample, containing up to 10 milliequivalents of primary amine, plus 3 ml. of benzaldehyde are heated for 30 minutes at 60 ± 1°C. After cooling to room temperature spontaneously, about 0.2 gram of sodium cyanide and 30 ml. of hydrogen cyanide reagent are added. The mixture is shaken vigorously for about 1 minute, allowed to stand at room temperature for 45 minutes, and then titrated with Karl Fischer reagent.

The blank contains 5 ml. of pyridine, 3 ml. of benzaldehyde, 0.2 gram of sodium cyanide, and 30 ml. of hydrogen cyanide reagent.

Free water in the sample is determined on a suitable aliquot after dilution with an appropriate known volume of acetic acid.

6. Determination of Anhydrides

a. Acid Hydrolysis

$$(RCO)_2O + H_2O \xrightarrow[CH_3COOH]{BF_3} 2\,RCOOH \qquad (6)$$

Reagents. (1) Hydrolysis reagent: 210 ml. of $BF_3 \cdot 2\,CH_3$-$COOH$ + 6.5 ml. of water diluted to 1 liter with acetic acid.

(2) Acetic acid.

(3) Pyridine.

Procedure. A 5 ml. aliquot of the sample in acetic acid solution, containing up to 4 millimoles of anhydride, plus 20 ml. of hydrolysis reagent are heated for 2 hours at 60 ± 1°C. After cooling to room temperature spontaneously, 5 ml. of pyridine are added and the solution is titrated with Karl Fischer reagent.

The blank contains 5 ml. of acetic acid, 20 ml. of catalyst, and 5 ml. of pyridine.

b. Alkaline Hydrolysis

$$(RCO)_2O + H_2O \xrightarrow[C_5H_5N]{NaI} 2\ RCOOH \qquad (7)$$

This technique should be applicable to acyl halides in general. Acetyl chloride and acetylsulfanilyl chloride are hydrolyzed with aqueous pyridine alone.

Reagents. (1) Hydrolysis reagent: 100 grams of sodium iodide plus 8 ml. of water diluted to 1 liter with pyridine.

(2) Pyridine.

Procedure. A 5 ml. aliquot of a pyridine solution of the sample, containing up to 10 milliequivalents of anhydride, plus 25 ml. of hydrolysis reagent are heated for 1 hour at 60 ± 1° C. After cooling to room temperature spontaneously, the solution is titrated with Karl Fischer reagent.

The blank contains 5 ml. of pyridine and 25 ml. of the hydrolysis reagent.

7. DETERMINATION OF CARBONYL COMPOUNDS

$$RCHO + H_2NOH{\cdot}HCl \longrightarrow RCH = NOH + HCl + H_2O \qquad (8a)$$

or

$$R_2CO + H_2NOH{\cdot}HCl \longrightarrow R_2C = NOH + HCl + H_2O \qquad (8b)$$

$$H_2NOH{\cdot}HCl + C_5H_5N{\cdot}SO_2 + C_5H_5N \longrightarrow H_2NSO_3H{\cdot}C_5H_5N + C_5H_5N{\cdot}HCl \qquad (9)$$

Reagents. (1) Hydroxylamine solution: 3.5 grams of $H_2NOH{\cdot}HCl$ diluted to 1 liter with methanol $\approx$ 0.5 normal.

(2) Pyridine-sulfur dioxide solution: 45 ml. of liquid SO_2 plus 80 ml. of pyridine in 875 ml. of methanol.

(3) Pyridine.

Procedure. The sample (weighed directly and containing up to 10 milliequivalents of carbonyl) plus 5 ml. of pyridine and 30 ml. of hydroxylamine reagent is heated for 2 hours at 60 ± 1°C. After cooling to room temperature spontaneously, 25 ml. of pyridine-sulfur dioxide solution are added. The solution is allowed to stand for 10 minutes (up to 1 hour does no harm) and then titrated with Karl Fischer reagent.

The blank contains 5 ml. of pyridine, 30 ml. of hydroxylamine reagent, and 25 ml. of pyridine-sulfur dioxide solution.

8. DETERMINATION OF CARBOXYLIC ACIDS

$$RCOOH + CH_3OH \xrightarrow{BF_3} CH_3COOR + H_2O \qquad (10)$$

This general procedure is applicable also to metal salts of organic carboxylic acids. In this case, however, because of their insolubility in dioxane, the samples are weighed directly into the flask.

$$RCOOM + CH_3OH \xrightarrow{BF_3} RCOOCH_3 + (BF_3)_x \cdot MOH \qquad (10a)$$

$$MOH + I_2 + SO_2 + CH_3OH \longrightarrow MI + HI + HSO_4CH_3 \qquad (10b)$$

or

$$RCOOM + CH_3OH \xrightarrow{BF_3} RCOOH + (BF_3)_x \cdot MOCH_3 \qquad (10c)$$

$$RCOOH + CH_3OH \longrightarrow RCOOCH_3 + H_2O \qquad (10d)$$

Reagents. (1) Esterification catalyst solution: 148 ml. of $BF_3 \cdot 2CH_3OH$ diluted to 1 liter with methanol ≎ 100 grams of BF_3 per liter.

(2) Dioxane, technical.

(3) Pyridine.

Procedure. A 5 ml. portion of a dioxane solution of the sample, containing up to 10 milliequivalents of acid (but not more than 15 milliequivalents of acid plus water), plus 20 ml. of esterification catalyst solution are heated for 1 hour at 60 ± 1°C. After cooling to room temperature spontaneously, 5 ml. of pyridine are added and the solution titrated with Karl Fischer reagent.

At least one blank, containing 5 ml. of dioxane, 20 ml. of catalyst, and 5 ml. of pyridine, is run with each set of samples.

9. DETERMINATION OF ESTERS

$$RCOOR' + KOH \longrightarrow RCOOK + R'OH \qquad (11)$$

This procedure also is applicable to a limited number of amides.

$$RCONH_2 + KOH \longrightarrow RCOOK + NH_3 \qquad (11a)$$

Reagent. (1) Alkali reagent: 14.5 grams of KOH dissolved in 100 ml. of diethylene glycol at 60°C., cooled to room temperature, and diluted to 250 ml. with more of the glycol ≎ 1 *N* KOH.

Procedure. The sample (weighed directly and containing up to 5 milliequivalents of ester plus acid) is transferred to a 250 ml. volumetric flask with a grooved glass stopper. Then 10 ml. of alkali reagent are added, the flask stopper is lubricated with high-temperature stopcock grease and sealed with a spring clamp. The flask is heated for 30 minutes at 125–130°C. After cooling to room temperature spontaneously, the contents are titrated with Karl Fischer reagent.

The blank contains 10 ml. of alkali reagent.

10. DETERMINATION OF NITRILES

$$RCN + H_2O \xrightarrow[CH_3COOH]{BF_3} RCONH_2 \qquad (12)$$

Reagents. (1) Catalyst solution: $BF_3 \cdot 2\,CH_3COOH$ containing 6.5 ml. of water per 500 ml. of solution.

(2) Pyridine.

(3) Acetic acid.

Procedure. A 5 ml. aliquot of an acetic acid solution of the sample, containing up to 20 milliequivalents of nitrile, plus 20 ml. of catalyst solution are heated for 2 hours at 80 ± 2°C. After cooling to room temperature spontaneously, 15 ml. of pyridine are added carefully and the solution is titrated with Karl Fischer reagent.

The blank contains 5 ml. of acetic acid, 20 ml. of catalyst solution, and 15 ml. of pyridine.

11. DETERMINATION OF DIALKYL AND HYDROPEROXIDES

$$ROOR + 2\,(H) \xrightarrow[H^+]{Zn} 2\,ROH \qquad (13a)$$

$$ROOH + 2\,(H) \longrightarrow ROH + H_2O \qquad (13b)$$

$$ROH + CH_3COOH \longrightarrow CH_3COOR + H_2O \qquad (14)$$

Reagents. (1) Catalyst solution: 210 ml. of $BF_3 \cdot 2\,CH_3COOH$ diluted to 1 liter with acetic acid $\approx$ 100 grams of BF_3 per liter.

(2) Zinc, powdered.

(3) Pyridine.

(4) Dioxane, purified.

Procedure. About 0.3 gram of zinc, the sample, containing up to 5 milliequivalents of peroxide, and 20 ml. of catalyst solution are placed in a 100 ml. glass-stoppered volumetric flask. The flask is connected immediately to a desiccant-protected reflux condenser and heated for 2 hours at 70 ± 5°C. After cooling to room temperature spontaneously, 5 ml. of pyridine are added carefully through the condenser followed by 5 ml. of dioxane. The contents are diluted to exactly 100 ml. with more dioxane, allowed to settle, and a 50 ml. aliquot is titrated with Karl Fischer reagent.

The blank contains an equal volume of the peroxide solvent, 0.3 gram of zinc, 20 ml. of catalyst, 5 ml. of pyridine, and sufficient dioxane to make 100 ml. A 50 ml. aliquot is titrated as above.

12. ANALYTICAL PROCEDURES APPLIED TO ANALYSIS OF COMPLEX MIXTURES

The analytical methods based on the use of Karl Fischer reagent have been employed for the evaluation of many complex organic mixtures. In most cases these analyses would have been impractical by other means, as illustrated by the following known compositions on which the components determined in duplicate by a Karl Fischer procedure are recorded in weight per cent:

No.	Composition	Added	Found	Procedure	Chapter
1	CH_3OH	33.2	33.2 ± 0.1	Hydroxyl	IX
	$HCOOCH_3$	57.5			
	$HCONH_2$	8.0			
	H_2O	1.3	1.3 ± 0.0		
	Total	100.0			
2	C_2H_5OH	48.7	48.5 ± 0.2	Hydroxyl	IX
	BF_3	43.2			
	HF	0.5			
	H_2O	7.6	7.7 ± 0.1		
	Total	100.0			
3	CH_3CH_2COOH	33.6	33.5 ± 0.2	Carboxyl	X
	$CH_3CH_2COOC_2H_5$	3.5			
	BF_3	55.7			
	HF	0.8			
	H_2O	6.4	6.4 ± 0.1		
	Total	100.0			
4	$CH_3(CH_2)_3OH$	6.2	6.4 ± 0.3	Hydroxyl	IX
	$C_2H_5COC_2H_5$	2.6			
	H_2O	91.2	91.1 ± 0.2		
	Total	100.0			
5	$CH_2(CH_2)_4CO$	15.5	15.5 ± 0.0	Carbonyl	XII
	$CH_2(CH_2)_4CHOH$	15.2			
	$CH_2(CH_2)_4CH_2$	18.7			
	CH_3COOH	49.9			
	H_2O	0.7	0.7 ± 0.0		
	Total	100.0			

TABLE (*concluded*)

No.	Composition	Added	Found	Procedure	Chapter
6	CH_3CHO	23.5	23.8 ± 0.1	Carbonyl	XII
	$(CH_3CO)_2O$	58.7			
	CH_3COOH	5.3			
	$CH_3COOC_2H_5$	12.5			
	Total	100.0			
7	$(CH_3CO)_2O$	1.2	1.2 ± 0.0	Anhydride	XI
	CH_3COOH	98.8			
	Total	100.0			
8	$H_2N(CH_2)_6NH_2$	28.2	28.5 ± 0.3	Primary amine	XIII
	$CH_3NH(CH_2)_6NHCH_3$	48.5	48.3 ± 0.2	Primary plus secondary amine	XIII
	$(CH_3)_2N(CH_2)_6N(CH_3)_2$	20.7			
	H_2O	2.6	2.4 ± 0.0		
	Total	100.0			
9	$NC(CH_2)_4CN$	16.3	16.3 ± 0.3	Nitrile	XIII
	$H_2NOC(CH_2)_4CONH_2$	43.7			
	$NC(CH_2)_4CONH_2$	29.3	29.0 ± 0.3	Nitrile	XIII
	$H_4NOOC(CH_2)_4COONH_4$	2.6			
	$HOOC(CH_2)_4COOH$	7.6			
	H_2O	0.5	0.4 ± 0.1		
	Total	100.0			
10	$(CH_3)_3COOC(CH_3)_3$	5.2	5.1 ± 0.2	Peroxide	XIV
	$(C_6H_5CO)_2O_2$	6.0			
	CH_3COOH	87.5			
	H_2O	1.3	1.3 ± 0.1		
	Total	100.0			

With the exception of the adiponitrile mixture (No. 9) these compositions were analyzed directly. Adiponitrile and cyanovaleramide were isolated from a mixture with the diamide, ammonium salt, and free acid by selective extraction with methylene chloride.

CHAPTER III

Nature of the Karl Fischer Reagent

A. Historical

Bunsen[1] demonstrated that at room temperature iodine, like bromine and chlorine reacts with water in the presence of sulfur dioxide according to the reaction:

$$I_2 + SO_2 + 2\,H_2O \longrightarrow 2\,HI + H_2SO_4 \qquad (1)$$

However, the reduction of iodine by sulfur dioxide in dilute acid solution reversed when the concentration of acid became greater than 0.05 per cent, the hydriodic acid being oxidized to free iodine:

$$H_2SO_4 + 2\,HI \longrightarrow I_2 + H_2SO_3 + H_2O \qquad (2)$$

In one of the first practical applications, Reich[2] utilized this reaction as the basis of an analysis for sulfur dioxide in kiln gases. The vapors were passed through a standard iodine in potassium iodide solution containing starch and continued until the starch indicator color just disappeared. Knowing the volume of gas involved and the amount of iodine in solution, he was able to calculate the concentration of sulfur dioxide in the gases. This technique received wide attention. Attempts to apply the method directly to the analysis for sulfur dioxide in gases formed in the lead chamber process for sulfuric acid manufacture failed because of the interference of nitrous acid, which reoxidized the hydriodic acid formed:

$$2\,HNO_2 + 2\,HI \longrightarrow I_2 + 2\,NO + 2\,H_2O \qquad (3)$$

Raschig[3] eliminated this difficulty by adding sodium acetate to the iodine solution. In this environment the sulfurous and nitrous acids reacted to form the corresponding sodium salts and acetic

---

[1] Bunsen, R., *Ann.*, 86, 265–91 (1853).

[2] Reich, F., *Die bisherigen Versuche zur Beseitigung des schädlichen Einflusses des Hüttenrauches bei den fiskalischen Hüttenwerken zu Freiberg*, Freiberg, 1858.

[3] Raschig, F., *Z. angew. Chem.*, 22, 1182–4 (1909).

acid. These salts did not interact and the iodine consumption, therefore, became a direct measure of sulfurous acid concentration.

Several other modifications later were made of Reich's original technique, particularly in apparatus design, but it was not until recently that the general reaction was made the basis of a new type of analysis, the quantitative determination of water. In 1935 Karl Fischer,[4] while employed in the laboratories of the Edeleanu Gesellschaft, described a reagent containing iodine and sulfur dioxide which was developed primarily for the determination of water in sulfur dioxide and then was found to be applicable to analyses for water in general. The specific nature of this reagent and the simplicity of its use soon were recognized by chemists. There is a continually increasing number of publications describing its application to the rapid analysis of all types of organic and inorganic compounds. The name "Karl Fischer Reagent" has become generally accepted in describing this solution.

In his initial experiments Fischer considered several techniques for ascertaining the extent of reaction, using benzene as solvent for the iodine and sulfur dioxide. Attempts to apply a starch endpoint were unsuccessful because of the failure of this indicator in a nearly anhydrous environment. Removal by distillation of the excess sulfur dioxide and the hydriodic acid formed in the reaction, followed by titration of the sulfuric acid produced, was unsuccessful; the consistently low results obtained were ascribed to previous reaction of the sulfuric acid with the benzene and to reduction of the product by the hydriodic acid.

Titration of the reagent with the sample to be analyzed for water until the solution was decolorized was found to be erratic, depending to a great extent on the concentration of the reagent. It was apparent also that, as the concentration of the acidic products increased, the reaction was reversed. In order to prevent reversal of the reaction it was necessary either to decompose the acid products or to introduce some material which would combine with them. The latter method appeared to be the more desirable. A study of the weak organic amines revealed that pyridine was particularly well suited to the purpose. It was found to have the additional advantage of combination with the sulfur dioxide, thereby reducing the

[4] Fischer, Karl, *Ibid.*, **48, 394–6 (1935).**

latter's vapor pressure. A tertiary amine thus became an essential component of the reagent. Reasoning by analogy to the completely inorganic system, Fischer suggested that the reaction proceeded as follows:

$$I_2 + 2\,H_2O + (C_5H_5N)_2{\cdot}SO_2 + 2\,C_5H_5N \longrightarrow (C_5H_5N)_2{\cdot}H_2SO_4 + 2\,C_5H_5N{\cdot}HI \quad (4)$$

The addition of the organic base rendered even less promising the titration of the apparent sulfuric acid formed in the reaction.

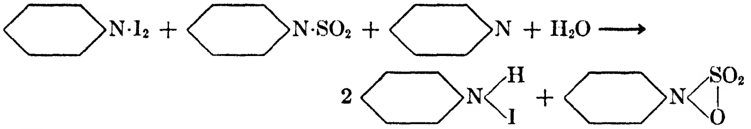
In addition to forming salts with the acidic products, the pyridine also combined irreversibly with sulfur dioxide (and hydriodic acid) on warming. Consequently, some of the compounds which interfered in the acidimetric titration could not be removed by distillation, thus preventing the selective determination of any sulfuric acid present.

By titrating with the pyridine-containing reagent until the iodine color was no longer discharged, i.e., until the first appearance of unused iodine, Fischer was able to discern a relatively sharp and reproducible endpoint. In order to insure a continuous excess of reactants other than iodine, he used three times the calculated quantity of sulfur dioxide and an additional excess of pyridine. His reagent consisted of 790 grams (10 moles) of pyridine, 192 grams (3 moles) of sulfur dioxide, and 254 grams (1 mole) of iodine dissolved in 5 liters of anhydrous solvent (benzene or methanol). This was calculated to be equivalent to 36 grams (2 moles) of water or to 7.2 milligrams of water per milliliter of reagent. (For the analysis of samples containing 0.1 per cent or less water an iodine solution diluted with solvent and equivalent to 1 or 2 milligrams of water per milliliter was employed.) The methanol solution of the above composition had the necessary properties of a successful reagent for the titrimetric determination of water.

B. Stoichiometry of the Reaction

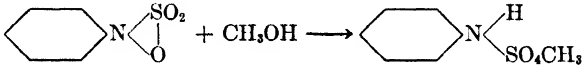
1. General

Fischer[4] has presented a good practical discussion of the preparation and use of his reagent but his suggested reactions do not adequately explain the observed quantitative results. (Although Fischer concluded that methanol was a more desirable solvent than benzene, it is likely that his conclusions concerning the reaction were based on initial studies in the hydrocarbon environment.) His reaction calls for the removal of 2 moles of water by each mole of iodine introduced, while actually only 1 mole or less of water is removed in the unmodified reagent. This was demonstrated by a thorough study of the stoichiometry of the Fischer method, including an investigation of the molar requirements of the various constituents (iodine, sulfur dioxide, pyridine, and methanol) and their relation to the removal of water.[5] This investigation revealed that Fischer's assumption that the reaction paralleled Bunsen's inorganic equation was incorrect. Actually the main reaction in methanol solution appears to take place in two distinct steps as follows:



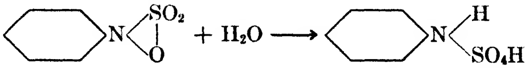
(5)

and:



(6)

Only equation (5) involves water absorption and the stoichiometric amount is only half that postulated by Fischer (equation 4). The intermediate compound, pyridine-sulfur trioxide, which is the "inner salt" of pyridinium hydroxide-*N*-sulfonic acid[6] can be isolated readily by omitting the methanol from the reagent (Table V and page 54). A modified reagent prepared from iodine, sulfur dioxide, and pyridine will absorb water approximating the 2 moles required by equation (4). In this case methanol in reaction (6) is replaced by 1 mole of water.



(7)

A reagent of this type is of little practical interest for the determination of water. It is not specific for water since reaction also takes place with alcohols (equation 6) and probably with other compounds containing active hydrogen. Also, reaction (7) may be used only indirectly since it is not accompanied by a positive color change although the indicator action of reaction (5) often can be

---

[5] Smith, D. M., Bryant, W. M. D., and Mitchell, J., Jr., *J. Am. Chem. Soc.*, 61, 2407–12 (1939).

[6] Baumgarten, P., *Ber.*, 59, 1166–71 (1926); 64, 1502–6 (1931); 64, 1582–9 (1931).

used to follow its course. Furthermore, the water equivalence varies greatly with minor differences of technique. Finally, in the absence of methanol the reagent soon discolors, changing from the normal deep brown to a deep green which is insensitive to the action of water.

The effect of the various constituents on reaction with water is illustrated in Table III. In these tests, freshly prepared solutions of iodine with sulfur dioxide in dimethylformamide (an inert solvent) were tested immediately or were first modified by the addition of excess pyridine or methanol. Solutions 1 and 4 could not be titrated directly. Excess water was added and the unused water determined by titration with standard Fischer reagent. This same technique was used for solution 2. The endpoint of solution 3, however, was determined approximately by titration with standard water solution to the first evidence of a marked color change.

TABLE III

Effect on Water Equivalence of Components of Fischer Reagent[a]

No.	Solution	Millimoles		Ratio (H_2O reacted to I_2 taken)
		Water reacted	Iodine taken	
1	$I_2 + SO_2$	65.1	33.45	1.95
2	$I_2 + SO_2 + C_5H_5N$	64.8	33.45	1.94
3	$I_2 + SO_2 + C_5H_5N$	36.7	33.45	1.10
4	$I_2 + SO_2 + CH_3OH$	30.6	33.45	0.91

[a] Conclusions but not data previously reported in literature reference 5.

Solutions 1 and 2 consumed water equivalent to about 2 moles per mole of iodine. In the experiment using solution 3 where the titration process was modified (that is, water was added in small increments to a fixed quantity of reagent), approximately an equimolar quantity of water had reacted when all of the iodine was reduced. Solution 4 (containing methanol) used up only about 1 mole of water per mole of iodine even though excess water was added. These experiments suggested that when pyridine or methanol are present in the reagent a two step process is involved, the first stage involving reaction with iodine, in support of equation (5), and the second step involving either water or methanol, in support of equations (6) and (7).

Further indication of reaction (6) and also of the stepwise reaction was obtained in several other experiments typified by the following, where individual 25 ml. solutions of iodine (33.3 millimoles) and sulfur dioxide (5 ml.) in pyridine, respectively, were freshly prepared and mixed. The solutions were titrated with standard water in pyridine until the deep iodine color was just discharged (first titer). After an equal amount of water was added, the excess was determined by direct titration with the normal Karl Fischer reagent, and the water consumed was calculated (second titer). The results are shown in Table IV.

TABLE IV

Reaction of Iodine and Sulfur Dioxide in Pyridine with Water in Absence and Presence of Methanol[a]

No.	Conditions	Water consumed,[b] millimoles			Iodine taken, millimoles	Ratio (H_2O reacted to I_2 taken)
		1st titer	2nd titer	Total		
1	2 hrs. at 60°C.[c]	36.6	29.6	66.2	33.3	1.99
2	1.5 hrs. at 28°C.[c]	38.3	27.3	65.6	33.3	1.97
3	2 min. at 28°C.[c]	38.3	27.3	65.6	33.3	1.97
4	25 ml. CH_3OH before 1st titer	25.1	−0.3	24.8	33.3	0.62
5	25 ml. CH_3OH after 1st titer but just before additional H_2O	43.1	−0.3	42.8	33.3	1.16
6	Additional H_2O after 1st titer immediately followed by 25 ml. CH_3OH	42.6	3.4	46.0	33.3	1.26

[a] Conclusions but not data previously reported in literature reference 5.
[b] Corrected for water in reagents.
[c] Time after mixing components to first titer.

Experiments 1 to 3, Table IV, indicate that over short periods of time the three component system (iodine–sulfur dioxide–pyridine) is thermally stable, since within the accuracy of the method the water equivalence was virtually unchanged. That a two step reaction is involved is supported by the effect of added methanol. When the alcohol was present initially the second step no longer used up water (experiment 4), in support of equation (6), but when additional water and methanol are added together (as shown in the last two experiments) both reactions (6) and (7) are in evidence as shown by the significant increase in water used up in experiment 6 over that of experiment 4.

2. MINIMUM SULFUR DIOXIDE REQUIREMENT

In order to establish the minimum sulfur dioxide requirement, two solutions were prepared, respectively, by dissolving 8.5 grams (33.5 millimoles) of iodine in 25 ml. of dry dimethylformamide and liquid sulfur dioxide in pyridine to form a 26 weight per cent solution, mixing the two solutions in varying ratios immediately before titrating with standard water in dimethylformamide just to the disappearance of the iodine color. No extra solvent was added. The experimental results are tabulated in Table V. The amount of sulfur dioxide solution corresponding to 33.5 millimoles of sulfur dioxide was about 8.3 ml.

TABLE V

Sulfur Dioxide Requirement in Karl Fischer Reagent[a]

SO_2 solution		Iodine taken, mM[b]	Water consumed, mM[b]	Ratio (H_2O reacted to I_2 taken)
Ml.	mM[b]			
25	102	33.5	36.8	1.10
15	61	33.5	36.8	1.10
10	40	33.5	37.5	1.12
5	20	33.5		No endpoint, solution very dark

[a] Conclusions but not data previously reported in literature reference 5.

[b] Millimoles.

The above data thus indicate the minimum $SO_2:I_2$ molar ratio to be 1:1.

3. MINIMUM PYRIDINE REQUIREMENT

To determine the required pyridine, 25 ml. of a 12 weight per cent solution of sulfur dioxide (47 millimoles) in dimethylformamide were mixed with 25 ml. of the iodine in dimethylformamide solution (33.5 millimoles of iodine). Varying amounts of pyridine were added and the solutions titrated with standard water in dimethylformamide solution until the brown color of the solution just failed to discharge. Experimental results are reported in Table VI.

With no pyridine present the solution was dark initially, changing to a bright orange in about 1 hour under the action of a large excess of water. Similar results were noted when 5 ml. and 6.3 ml. of pyridine were added. With 8 ml. (100 millimoles) or more, however, an orange endpoint developed immediately on direct titration indicating the minimum pyridine:iodine molar ratio to be 3:1.

TABLE VI

Pyridine Requirement in Karl Fischer Reagent[a]

Pyridine		Iodine taken, mM[b]	Water consumed, mM[b]	Ratio (H_2O reacted to I_2 taken)
Ml.	mM[b]			
25	310	33.5	38.4	1.15
10	126	33.5	38.8	1.16
8	100	33.5	39.5	1.18
6.3	80	33.5		No endpoint
5	63	33.5		No endpoint

[a] Conclusions but not data previously reported in literature reference 5.
[b] Millimoles.

4. MINIMUM METHANOL REQUIREMENT

The data given in Table VII were derived from experiments in which 25 ml. portions of iodine (31.5 to 33.5 millimoles) in pyridine were mixed with 10 ml. of 26 weight per cent sulfur dioxide (40 millimoles) in pyridine. Varying quantities of methanol were added and the mixtures titrated immediately with standard water in pyridine to the discharge of the iodine color.

TABLE VII

Methanol Requirement in Karl Fischer Reagent[a]

Methanol		Iodine taken, mM[b]	Water consumed, mM[b]	Ratio (H_2O reacted to I_2 taken)	Remarks[c]
Ml.	mM[b]				
50	1250	33.3	25.1	0.75	Clear yellow
20	500	33.5	25.0	0.75	Clear yellow
10	250	31.5	24.0	0.76	Clear yellow
5	125	31.5	24.0	0.76	Clear yellow
2	50	33.5	25.4	0.76	Clear yellow
1	25	31.5	26.5	0.84	Fairly dark
0	0	33.5	37.0	1.10	Dark

[a] Conclusions but not data previously reported in literature reference 5.
[b] Millimoles.
[c] Color of endpoint on titration of reagent with water in pyridine solution.

Although the absence of methanol did not prevent the removal of the deep brown iodine color by water, an appreciable change in the actual amount of water absorbed and in the color of the endpoint occurred as the molal ratio of methanol to iodine was reduced to less than 1, while above this ratio only minor differences were observed. In the absence of methanol the water absorbed at the characteristic endpoint varied from about 1 mole when water was added to the reagent to about 2 moles when the process was reversed (see Tables III and IV). In the presence of 1 mole or more of methanol between 0.7 and 0.8 mole of water was absorbed per mole of iodine.

In a reagent of the preferred composition the water equivalence remains constant regardless of the order of mixing of the components, as shown by the following series of experiments. In the first case iodine in pyridine was mixed with varying excesses of methanol. After the addition of excess sulfur dioxide, the water consumed on titration varied from 0.7 to 0.8 mole of water per mole of iodine. The same water equivalence was noted: when iodine was added to sulfur dioxide in pyridine, followed by excess methanol; when sulfur dioxide in pyridine was added to iodine in methanol; when pyridine, iodine, and methanol were mixed and sulfur dioxide added; and when a solution of pyridine, iodine, and sulfur dioxide was prepared and methanol added.

In addition to supporting the reactions proposed in equations (5–7), the foregoing observations indicate that the stoichiometric ratios for the alcoholic reagent are $I_2 : SO_2 : 3C_5H_5N : CH_3OH$ as opposed to Fischer's equation (4) which requires 4 moles of pyridine and no methanol.

Having established the minimum ratios of the constituents of the Fischer reagent, it was deemed advisable to determine the identity of the crystalline substances in active and "spent" solutions. This was accomplished by the comparison of optical crystallographic constants determined on likely, known, crystalline compounds and on substances isolated from the reagent under various conditions.

C. Addition Compounds Involved in Karl Fischer Reagent

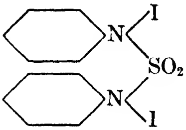
1. LIKELY BINARY COMPOUNDS IN THE ACTIVE REAGENT

The literature gives little or no indication of the chemical nature of solutions of iodine and sulfur dioxide in pyridine. It was first believed that the active constituents of the reagent were present as a complex compound of pyridine with sulfuryl iodide analogous to acetylpyridinium chloride formed from acetyl chloride and pyridine.

According to Mellor, however, iodine and sulfur dioxide do not combine appreciably to form sulfuryl iodide:[7]

$$I_2 + SO_2 \longrightarrow SO_2I_2 \tag{8}$$

In the organic environment of the reagent, this iodide might possibly be involved in the mechanism, although up to the present time there has been no evidence found in support of such a combination—for example, a ternary,



When either iodine or sulfur dioxide is dissolved in pyridine a considerable amount of heat is evolved. On mixing these two combinations, however, the temperature change is negligible. Furthermore, a solution of equimolar quantities of iodine and sulfur dioxide in excess pyridine, when partially evaporated, precipitated crystals identified optically as pyridine diiodide with some pyridine-sulfur trioxide and pyridine hydroiodide, the latter two from the reaction with the small amount of water in the pyridine.

Binary compounds of pyridine with both iodine and sulfur dioxide have been reported in the literature but accounts of their composition have varied. Carlsohn[8] reported a binary $C_5H_5N{\cdot}I_2$ (M.P. 62°C.) and also a pyridine-rich compound $(C_5H_5N)_2{\cdot}I_2$ (M.P. 53°C). Chatelet[9] reported on the isolation of a compound $(C_5H_5N)_2{\cdot}I_2$. In the presence of water, however, he proposed a hydrate $C_5H_5N{\cdot}I_2{\cdot}6H_2O$. Only one binary compound has been isolated by the authors where iodine was dissolved in excess pyridine in an anhydrous environment. Pyridine diiodide, $C_5H_5N{\cdot}I_2$, was prepared in this laboratory by the rapid evaporation of a solution of 0.05 mole of iodine in about 0.4 mole of pyridine. The orange colored crystals of the diiodide were not very stable, particularly in a moist atmosphere where they first partially liquefied and then lost pyridine leaving a dark greenish crystalline material. They were

---

[7] Mellor, J. W., *A Comprehensive Treatise on Inorganic and Theoretical Chemistry*. Vol. X, Longmans, Green, London, 1930, p. 676.

[8] Carlsohn, H., *Ueber eine neue Klasse von Verbindungen des positiv einwertigen Iods.*, S. Hirzel, Leipzig, 1932.

[9] Chatelet, M., *Ann. Chim.*, [11], 2, 5–25 (1934).

fairly stable under paraffin hydrocarbons, Nujol, and, surprisingly, water. The pyridine diiodide crystals were dissolved by benzene; on treating the solution with an equal volume of dry hexane, the original crystalline derivative was immediately reprecipitated. This compound was precipitated, also, on the slow addition with stirring of an equal volume of water to a 20 per cent solution of iodine in pyridine. Too rapid addition of water imparted a red turbidity to the solution, probably indicating partial decomposition. Analysis of crystals from hexane precipitation after most of the hexane had been removed in a current of dry air (complete drying encouraged decomposition) indicated 75.5 per cent iodine by titration with thiosulfate, corresponding favorably with a calculated value of 76.3 per cent iodine in $C_5H_5N{\cdot}I_2$.[5] $(C_5H_5N)_2{\cdot}I_2$ would contain 61.6 per cent iodine and $(C_5H_5N)_2{\cdot}I_3$, 70.7 per cent.

The dark greenish crystalline solid observed after exposure of the diiodide to air melted at about 85°C. and analyzed 84.9 per cent iodine; the compound $C_5H_5N{\cdot}I_4$ would contain 86.5 per cent iodine. A "periodide" of this type has been described,[10] having been obtained after mixing a 15 per cent solution of iodine in absolute ethanol with a few milliliters of dry pyridine.

The composition of the pyridine-sulfur dioxide binary compound has been formulated both as $C_5H_5N{\cdot}SO_2$[11] and $(C_5H_5N)_2{\cdot}SO_2$.[4] Recently, Hoffman and VanderWerf[12] reported on melting point–composition data for the system pyridine-sulfur dioxide which confirmed in detail that only the single stable compound, $C_5H_5N{\cdot}SO_2$, exists in this binary system (Fig. 1).

Their melting point of —7.4°C. for this compound appears to be slightly low on the basis of the previously reported value of —4.4° to —4.8°C.[5]

Observations in the authors' laboratory of the melting points of mixtures of pyridine and sulfur dioxide in the vicinity of these compositions disclosed a 1 : 1 compound melting sharply at —4.4° to —4.8°C. while the 2 : 1 mixture melting over the range —11° to —50°C. was obviously not homogeneous. Similarly 1:2 and 0.8:1 mixtures melted over the ranges —35° to —75°C. and —9° to —14°C., respectively. When the pyridine-sulfur dioxide compound was cooled in solid carbon dioxide–methanol and then gradually warmed, an ap-

[10] Prescott, A. B., and Trowbridge, P. F., *J. Am. Chem. Soc.*, 17, 859–69 (1895).

[11] André, M. G., *Compt. rend.*, 130, 1714–16 (1900).

[12] Hoffman, K. R., and VanderWerf, C. A., *J. Am. Chem. Soc.*, 68, 997–1000 (1946).

preciable quantity of crystalline material remained at room temperature. This same effect was noticed after the addition of up to 5 mole per cent water. These crystals, most likely a polymeric form of $C_5H_5N{\cdot}SO_2$, appeared to be identical with those described by André.[11] Other reported complexes in which 1 mole of sulfur dioxide is combined with 1 mole of an amine include $C_6H_5N(CH_3)_2{\cdot}SO_2$,[13] $(C_3H_7)_3N{\cdot}SO_2$,[14] $(C_5H_{11})_3N{\cdot}SO_2$,[15] and $CH_3C_5H_4N{\cdot}SO_2$.[12] The α- and γ-picolines also form the stable compounds, $2CH_3C_5H_4N{\cdot}3SO_2$ and $CH_3C_5H_4N{\cdot}2SO_2$, respectively.[12]

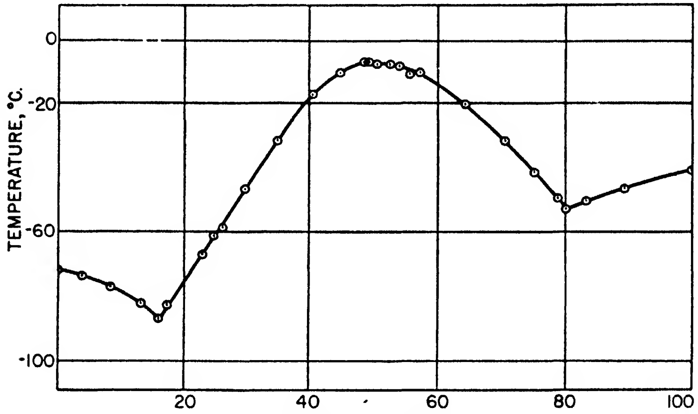
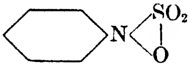


Fig. 1. Melting point–composition data for the system sulfur dioxide-pyridine.[12]

Pyridine-sulfur trioxide:



(I)

was prepared both by the dropwise addition of pyridine to fuming sulfuric acid and by the method of Baumgarten:[6]

$$K_2SO_4{\cdot}SO_3 + C_5H_5N \longrightarrow C_5H_5N{\cdot}SO_3 + K_2SO_4 \qquad (9)$$

This compound is not very stable. It is converted to pyridine hydrogen sulfate in the presence of hot water and to pyridine hydrogen methyl sulfate by methanol at room temperature.

According to Baumgarten[6] the reaction of potassium pyrosulfate with amines aparently is general:

---

[13] Bright, J. R., and Jasper, J. J., *Ibid.,* 63, 3486–8 (1941).

[14] Jasper, J. J., and Bright, J. R., *Ibid.,* 66, 105–6 (1944).

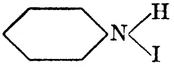
[15] Bateman, L. C., Hughes, E. D., and Ingold, C. K., *J. Chem. Soc.,* **1944,** 243–7.

$$K_2SO_4 \cdot SO_3 + 2\ NH_3 \longrightarrow NH_2 \cdot SO_3(NH_4) + K_2SO_4$$
$$K_2SO_4 \cdot SO_3 + R_3N \longrightarrow R_3N \cdot SO_3 + K_2SO_4$$

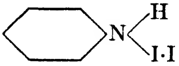
2. Likely Compounds in "Spent" Fischer Reagent

A number of pyridine compounds which might appear in spent Fischer reagent were prepared and classified. Pyridine hydroiodide crystals (II) were deposited on mixing equal volumes of molar hydroiodic acid in methanol with molar pyridine in methanol. The crystals were stable and apparently nonhygroscopic.

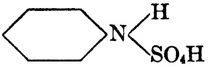
Pyridine hydroperiodide (III) was prepared from pyridine hydroiodide and iodine according to the method of Trowbridge.[16]



(II)



(III)



(IV)

Pyridine hydrogen sulfate (IV) was recovered when 10 millimoles of concentrated sulfuric acid were mixed with 20 millimoles of pyridine and the resulting clear, colorless solution heated gently on a hot plate in the presence of xylene (to aid in removing the water) until the temperature reached about 160°C. Pyridine was lost and, on cooling, a solid crystalline cake was formed. This was shown to contain equimolar quantities of sulfuric acid and pyridine by titration to phenolphthalein to determine sulfuric acid, followed by back titration to bromophenol blue to determine pyridine.[5] Peakin[17] made the surprising observation that electrometric titration of a molar aqueous solution of pyridine with normal aqueous sulfuric acid gave an inflection corresponding to $(C_5H_5N)_2 \cdot H_2SO_4$ but none for $C_5H_5N \cdot H_2SO_4$. On the other hand, his study of the ternary system pyridine–sulfuric acid–water at 20°C. showed that the 1 : 1 compound is the only stable solid phase. In the latter case various combinations of the three components were placed in a water bath for 2 hours at 20°C. If a solid separated, the liquid was sampled and analyzed for sulfuric acid by titration with alkali to phenolphthalein and for pyridine by back titration with sulfuric acid to bromophenol blue. The composition of the solid phase then was deduced from the tie-line construction on a triangular diagram. Where two liquid phases were present, portions of each were analyzed by titration.

[16] Trowbridge, P. F., *J. Am. Chem. Soc.*, 19, 322–31 (1897).
[17] Peakin, F. H., *J. Soc. Chem. Ind.*, 59, 56–7 (1940).

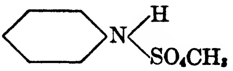
TABLE VIII

Optical Properties and Melting Points of Pyridine Derivatives[5]

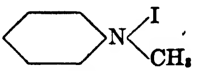
Compound	M.P., °C.[a]	Optic axial angle[b,c]	Optical character	Dispersion	Miscellaneous observations
$C_5H_5N \cdot I_2$	62	2 Ha = 59 ± 1° 2 E = 96.5 ± 1°	+	r>v, moderate; distinct horizontal dispersion	Orange plates elongation $\parallel \alpha$
$C_5H_5N \cdot I_4$	85				Dichroism yellow to black, extinction angle = 10 ± 2°
$C_5H_5N \cdot HI$	231 (slow dec. at 195)	Uniaxial	−		Compact colorless or pale yellow crystals
$C_5H_5N \cdot HI \cdot I$	193–195	Uniaxial	+		$\omega + \epsilon > 1.75$, pleochroism yellow $\parallel \omega$, red-brown to black $\parallel \epsilon$
$C_5H_5N \cdot CH_3I$ (A)[d]	119	2 Ha = 65 ± 1° 2 Ho = 140 ± 10°	−	r>v, moderate	Elongation (Bxo) $\wedge \beta$ 32 ± 2°
$C_5H_5N \cdot CH_3I$ (B)	121	2 Ha = 90 ± 1°	−	r>v, strong	Elongation (Bxo) $\parallel \gamma$
$C_5H_5N \cdot H_2SO_4$	101–105	2 Ha = 51 ± 1°	−	r>v, moderate	$\alpha = 1.430^c \pm 0.005$, $\beta = 1.615 \pm 0.005$
$C_5H_5N \cdot SO_3$		2 Ho = 135 ± 10°	−		$\alpha = 1.470 \pm 0.003$, $\beta = 1.690 \pm 0.003$
$C_5H_5N \cdot HSO_4CH_3$	112–117	2 Ha = 24 ± 1° 2 Ho = 135 ± 5°	−	r>v, moderate	Needles and plates elongation $\parallel \beta$

[a] Corrected melting points by Kofler-Hilbck microheating stage.
[b] For details regarding this constant see Bryant.[19]
[c] Optic axial angle and refractive indices referred to 5461Å Hg line at 25 ± 3°C.
[d] Stable modification.

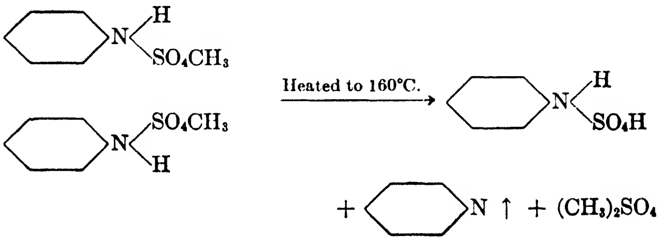
Pyridine hydrogen methyl sulfate (V) was isolated after evaporation of a benzene solution of slightly more than 0.1 mole of pyridine with 0.1 mole of sulfuric acid in methanol. The glistening white plates which precipitated were stable in air and not appreciably hygroscopic. Pyridine hydrogen methyl sulfate, when heated above its melting point, gradually lost vapor up to about 150°C. but showed no further tendency to boil at 160°C. On cooling, crystals identified as pyridine hydrogen sulfate formed in a syrupy mother liquor, presumably through the reaction:



(V)



(VI)



(10)

Methylpyridinium iodide (VI) was prepared from pyridine and methyl iodide by the method of Prescott.[18] On recrystallization from hot absolute ethanol the white crystals melted sharply at 119°C. on slow heating, but just before fusion was complete another crystalline phase was formed which melted at 121°C. The two modifications apparently were true polymorphs, the lower melting being a stable modification.

3. IDENTIFICATION OF CRYSTALLINE CONSTITUENTS IN ACTIVE AND SPENT KARL FISCHER REAGENT

Sufficient physical data were obtained on known compounds to insure identification if these crystalline materials should be isolated from active and spent reagent. These constants are recorded in Table VIII.

The crystalline compounds were isolated from active and spent modified Fischer reagent, containing pyridine, iodine, and sulfur dioxide, but no methanol, after evaporation of the liquid *in vacuo*

---

[18] Prescott, A. B., *J. Am. Chem. Soc.,* **18,** 91–6 (1896).

followed by desiccation under nitrogen. In the active reagent pyridine diiodide was the principal compound found, along with some pyridine hydroperiodide, pyridine-sulfur trioxide, pyridine hydrogen sulfate, and an unknown ($2Ha = 28 \pm 10°$, positive, $r > v$).[19] The presence of pyridine-sulfur trioxide, $C_5H_5N \cdot SO_3$, supported the proposed stepwise reaction (5–6). Appreciable quantities of this compound were isolated when a mixture of iodine, sulfur dioxide, and pyridine in the stoichiometric ratios was poured slowly onto chopped ice and stirred to prevent local overheating. In another experiment, on pouring a strong Fischer reagent, containing 10 moles of methanol per mole I_2, onto ice the authors[5] were able to recover and identify the intermediate pyridine-sulfur trioxide. In this case, also, identity was based on comparison of optical crystallographic measurements with those of the known materials.

In the normal spent reagent, obtained by adding sufficient water to just discharge the iodine color, pyridine hydroiodide and pyridine hydrogen methyl sulfate were the principal crystalline components together with small quantities of pyridine hydrogen sulfate and two unidentified materials ($2\ Ha = 38°$, negative, and $2\ Ha = 43°$, positive). Further proof of the absence of any appreciable quantity of pyridine hydrogen sulfate was obtained by treating spent Fischer reagent with acid barium chloride solution; in the cold only a slight turbidity resulted. After boiling spent Fischer reagent with concentrated hydrochloric acid, however, a heavy precipitate formed immediately on adding barium chloride to the cooled mixture. This was rather conclusive evidence that practically all of the sulfate in spent reagent was present as methyl sulfate ion, $SO_4CH_3^-$, which was hydrolyzed by strong acid slowly in the cold but rapidly on heating.

D. Nature of Parasitic Side Reactions

While equations (5) and (6) fix the maximum absorption of water by the regular reagent at 1 mole per mole of iodine, this theoretical efficiency is rarely attained because of side reactions. Including the correction for the water in the components, the freshly prepared reagent usually is equivalent to about 80 per cent of the theoretical, but in the course of about a month its strength falls to about 50 per cent, and in three months to about 40 per cent, of

---

[19] Bryant, W. M. D., *Ibid.*, 60, 1394–9 (1938).

the theoretical. This is shown graphically in Figure 2 in which are plotted the results obtained when freshly prepared reagent was standardized at regular intervals by titration of a known quantity of water in methanol. Initial titrations were made within 15 minutes after addition of the sulfur dioxide at which time, after allowing for known water initially present, the reagent was equivalent to 84, 81, and 86 per cent of theory in curves A, B, and C, respectively. The uppermost curve represents the complete reagent freshly prepared while the two lower curves represent stored stock solution (iodine and pyridine in methanol) to which sulfur dioxide was added just before the initial titration. Similar observations were made by Almy, Griffin, and Wilcox[20] who found that the water equivalence of their reagent was never greater than 35 per cent of that called for by Karl Fischer's equation.

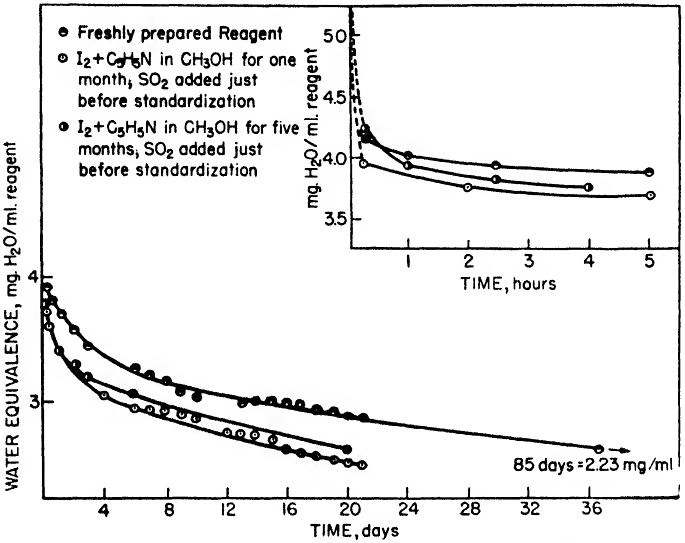


Fig. 2. Rate of degradation of Karl Fischer reagent.

Carlsohn[8] has indicated that iodine is used up by pyridine, possibly through the formation of an iodopyridine. The consumption

---

[20] Almy, E. G., Griffin, W. C., and Wilcox, C. S., *Ind. Eng. Chem., Anal. Ed.*, **12**, 392-6 (1940).

of iodine was verified in this laboratory although the extent of reaction was considerably less than that reported by Carlsohn. In one case a one normal solution of iodine in pyridine was held at room temperature. At the indicated intervals, portions were titrated for free iodine with standard aqueous thiosulfate:

Elapsed time from preparation of solution	Per cent of total iodine present	Elapsed time from preparation of solution	Per cent of total iodine present
0.25 hour	100.0	23 hours	94.2
0.5 "	99.2	47 "	93.4
6 hours	97.6	15 days	87.9

In the absence of methanol from a reagent containing sulfur dioxide and iodine in pyridine, both pyridine and total acidity decreased on standing. From such a freshly prepared (methanol free) reagent, which contained the ingredients in the usual proportions and which had sufficient water added to it to discharge the iodine color, 97 per cent of the pyridine was recovered by distillation up to 100°C. from alkaline solution. The pyridine, recovered as the water azeotrope, was determined by acidimetric titration to bromphenol blue. In order to show that no other volatile base (for example, iodopyridine) was present in the distillate, a picrate was made of the last cut. The optical crystallographic constants of this derivative were in exact agreement with those of pyridine picrate.[21] After standing for 1 month, similar treatment of the modified reagent accounted for only 71 per cent of the pyridine, indicating irreversible side reactions, possibly including derivatives of iodopyridine. A considerable amount of high boilers in the form of brown tarry material remained as part of the distillation residue.

The presence of excess methanol apparently had a marked effect on the course of these side reactions as indicated by the following experiments. Freshly prepared standard Fischer reagent containing 28.2 grams of iodine, 88 grams of pyridine, 220 ml. of dry methanol, and 15 ml. of liquid sulfur dioxide was immediately discharged with a minimum quantity of water (1.7 ml.). The solution was cooled, a solution of 35 grams of sodium hydroxide in 250 ml. of water added, and the mixture distilled. Over 99 per cent (219 ml.) of the methanol and 97 per cent (85 grams) of the pyridine were

---

[21] Mitchell, J., Jr., and Bryant, W. M. D., *J. Am. Chem. Soc.,* **65, 128–37 (1943).**

recovered. A similar reagent (160 ml.) to which sulfur dioxide had been added three months previously was discharged with water and treated as above. About 93 per cent (102 ml.) of the added methanol and 90 per cent (38 grams) of the added pyridine were recovered.

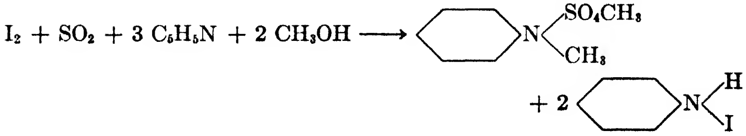
In the steam distillation of the aged Fischer reagent, as the residue became more concentrated, a small amount of distillate with a very sharp amine odor somewhat like piperidine was obtained. This liquid was alkaline; it reduced potassium permanganate readily but did not form a crystalline picrate. Apparently the same volatile base was produced when methylpyridinium iodide was distilled with aqueous sodium hydroxide; hence it has been assumed that the methylpyridinium ion is present in aged Fischer reagent.[5] The unknown base was probably an *N*-alkyldihydropyridine since these compounds are known to form when alkylpyridinium salts are distilled with strong caustic.[22] Thus, methanol apparently discourages any tendency toward iodination of the pyridine. This is supported by the fact that the effective strength of the reagent showed no appreciable change with time when stored in the absence of sulfur dioxide (Fig. 2). In one case (lowest curve), the solution of iodine and pyridine in methanol, stored five months before the addition of the usual quantity of sulfur dioxide, showed approximately the same water equivalence as that given by freshly prepared stock solution. Then, too, essentially all of the iodine was recoverable as iodide ion regardless of standing time. Therefore, Fischer's assumption[4] that the reagent, when stored in a tightly stoppered flask, loses strength through substitution of hydrogen in pyridine by iodine is unlikely on the basis of the above experiments. Also there is no evidence of iodine addition.

A comparison of these studies with those conducted in the absence of methanol definitely indicated that in the regular Fischer reagent the principal endproducts of the side reactions are hydrolyzed in alkaline solution, releasing substantially all methanol and pyridine originally present.

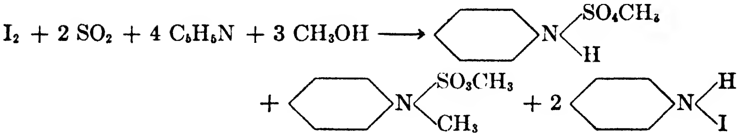
The side reactions for the methanol reagent have been found to include the reduction of iodine to the iodide ion and the formation of quaternary methylpyridinium salts,[5] probably as follows:

---

[22] Meyer, V., and Jacobson, P., *Lehrbuch der organischen Chemie*. Vol. II, Part 3, de Gruyter, Berlin, 1920, p. 799.



(11)



(12)

The types of impurities in the pyridine had a decided effect on the rate of degradation of Fischer reagent. Almy, Griffin, and Wilcox[20] noted that some grades of pyridine were inferior as evidenced by decreased stability of the reagent. Keating and Scott[23] found that a reagent made with a practical grade of pyridine of wide boiling range indicated less degradation on standing than one made with C.P. pyridine obtained from the same source. Their data, plotted in Figure 3, show that the decrease in titer was not uniform since deviations from a smooth curve were considerably larger than the probable experimental errors. (The numerical differences between the curves cannot be taken as absolute since the rate of degradation is not exactly reproducible for successive preparations using ingredients of the same purity.)

An unusual case of little or no degradation of Fischer reagent with time has been reported by Aepli and McCarter.[24] Their data, shown in Figure 4, were obtained on three different lots of commercially available Fischer reagent, two of which showed the usual slow but steady decomposition. The marked stability of the one preparation apparently has not been observed with any other laboratory prepared solutions or samples of the commercial reagent.

In general the rate of decomposition of Fischer reagent decreases rapidly after the first several hours. Consequently it has been recommended that the reagent be prepared 24 hours or more prior to actual use.[20,24] Where less time is allowed, frequent standardization of the reagent is necessary.

---

[23] Keating, J. F., and Scott, W. M., *Am. Dyestuff Reptr.*, **31**, **No. 13**, *Proc. Am. Assoc. Textile Chem. Colorists*, 308–10 (1942).

[24] Aepli, O. T., and McCarter, W. S. W., *Ind. Eng. Chem., Anal. Ed.*, **17**, **316–7** (**1945**).

There has been no evidence that this deterioration is accelerated by increased temperature. Heating the reagent at 60–70°C. for a few minutes apparently has no effect. On cooling, the color is discharged normally when the reagent is titrated with water; a reagent equivalent to 0.181 millimole of water per milliliter gave a value of 0.180 after heating. In some cases Fischer reagent has been heated at 50–60°C. to aid in the extraction of moisture from insoluble materials (see Chapter VI). The reagent also has been

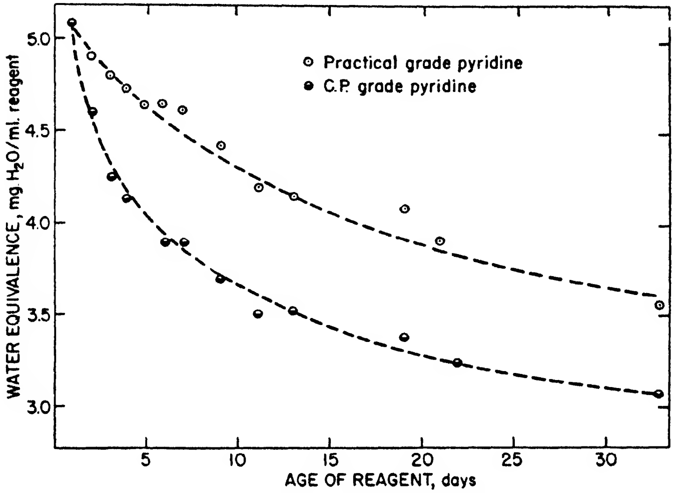


Fig. 3. Change of titer of Karl Fischer reagent with age.[23]

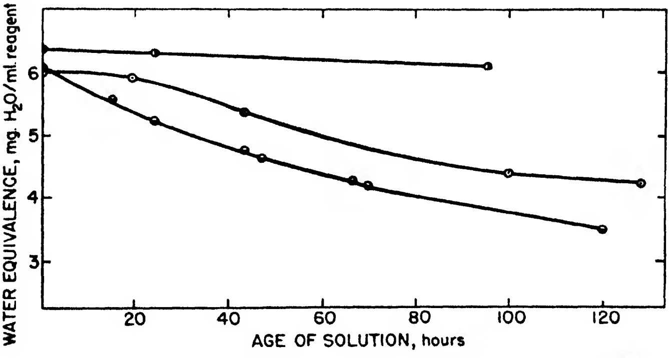


Fig. 4. Decomposition of commercial Fischer reagent.[24]

used effectively at reduced temperatures. As demonstrated in Chapters V and VI, titrations were made successfully at temperatures as low as — 60°C.

Karl Fischer reagent, in which the iodine has just been discharged, tends to darken when allowed to stand in a closed dry system. Apparently iodine is slowly regenerated under these conditions, through oxidation of the iodide ion, since on the addition of more water, the color is again discharged. For most purposes this effect is negligible, as shown in Table IX. Samples of methanol and pyridine were titrated to a positive endpoint, sufficient water in pyridine added to just discharge the iodine color, and the sealed flasks allowed to stand as indicated. After the given time interval the solutions were back titrated with standard water in pyridine.

TABLE IX

Reappearance of Iodine in 25 Milliliters of Spent Fischer Reagent

Sample	Conditions	Time, hours	Ml. of water in pyridine to return to endpoint
Methanol, 50 ml.	5°C., dark	18	
	25°C., dark	18	
	25°C., light	18	0.3
	60°C., light	18	0.6
Pyridine, 25 ml.	5°C., dark	18	No change
	25°C., dark	18	0.05
	25°C., light	18	0.1
	60°C., light	18	0.15

E. Studies on Modifications of Karl Fischer Reagent

1. General

Several other nitrogen bases have been tested as substitutes for pyridine and other solvents in place of methanol. Karl Fischer[4] stated that aniline may be used in place of pyridine, and benzene for methanol. He observed, however, that in the hydrocarbon environment the endproducts often were insoluble and tended to obscure the endpoint. Zavarov,[25] unable to prepare sufficiently dry methanol, found that ethanol could be used satisfactorily.

---

[25] **Zavarov, G. V.,** *Zavodskaya Lab.,* **5, 1314–8 (1936).**

2. POSSIBLE SUBSTITUTES FOR PYRIDINE

In a more thorough study the authors with W. M. D. Bryant tried a variety of organic amines as substitutes for pyridine, most of which have been reported in the literature.[5] Contrary to Fischer's observation, it was found that aniline was unsatisfactory; a reagent employing aniline decomposed almost completely in 24 hours. Morpholine and triethanolamine encouraged decomposition of the reagent during preparation. Quinoline and tributylamine were satisfactory but in the case of the aliphatic base apparently 4 moles could be required to effect discharge of the reagent as opposed to the necessary 3 moles of pyridine (see Tables VI and X). Dimethylaniline was somewhat better than aniline but the reagent still was relatively unstable. These conclusions were based on titration of known quantities of water in methanol with a reagent consisting of 42.3 grams of iodine, 125 ml. of dry nitrogen base, and 25 ml. of liquid sulfur dioxide diluted to 250 ml. with dry methanol. A corresponding pyridine solution was used as a control.

TABLE X

Effect of Organic Nitrogen Bases in Modified Karl Fischer Reagent[a]

No.	Base	Quantity		Iodine taken, mM[b]	Water consumed, mM[b]	Ratio (H_2O reacted to I_2 taken)
		Ml.	mM[b]			
1	C_5H_5N	25	305	33.3	33.4	1.00
2	C_5H_5N	25	305			
	CH_3OH	2	50	33.3	27.3	0.82
3	$C_6H_5{\cdot}NH_2$	25	280	33.3	15.3[c]	0.46
4	$C_6H_5{\cdot}NH_2$	15	160	33.3	20.6[c]	0.62
5	$C_6H_5{\cdot}NH_2$	12.8	138	33.3	23.3	0.69
6	C_5H_5N	25	305	Sample decolorized before addition of water		
	$C_6H_5{\cdot}NH_2$	15	160			
7	C_9H_7N[d]	25	195	No endpoint		
8	C_9H_7N	25	195			
	CH_3OH	25	625	33.3	34.4	1.03
9	$C_6H_5{\cdot}N(CH_3)_2$	25	205			
	CH_3OH	25	625	33.3	23.1	0.69
10	$(C_4H_9)_3N$	50	240			
	CH_3OH	50	1250	33.3	24.7	0.74
11	$(C_4H_9)_3N$	25	120			
	CH_3OH	25	625	33.3	22.2	0.67
12	$(C_4H_9)_3N$	15	73	No endpoint		
	CH_3OH	25	625			

[a] Conclusions but not data previously reported in literature reference 5.
[b] Millimoles.
[c] Turbid yellow endpoint, all others clear. [d] Quinoline.

A second series of experiments was designed to eliminate any possible influence of the methanol. A weighed quantity of iodine (8.45 grams, 33 millimoles) and 6.3 ml. of liquid sulfur dioxide were each dissolved in 25 ml. of dimethylformamide. These solutions were mixed, a known volume of base added, and the final reagent titrated immediately with a standard solution of water in dimethylformamide. Comparative data with and without methanol present are given in Table X.

Aniline in molar excess over iodine of greater than about 4 to 1 tended to precipitate solids in the Fischer reaction, as evidenced by the turbid endpoint when 15 ml. or more of this base were present. No endpoint could be discerned when quinoline alone was used, but in the presence of an equal volume of methanol the usual clear yellow endpoint was obtained.

3. Possible Substitutes for Methanol

A wide series of solvents was tried as possible substitutes for methanol. The principal disadvantage of most of these when incorporated in the reagent was their failure to dissolve the amine salts produced by the action of water, obscuring the endpoint to a serious degree.

TABLE XI

Effect of Solvents in Karl Fischer Reagent[a]

Auxiliary solvent	Quantity		Iodine taken, mM[b]	Water consumed, mM[b]	Ratio (H_2O reacted to I_2 taken)
	Ml.	mM[b]			
C_2H_5OH	50	870	33.5	33.3	1.00
C_2H_5OH	2	35	33.5	27.2	0.81
CH_3CN	50	980	33.5	38.6	1.15
CH_3CN	2	48	33.5	36.5	1.09
CH_3OH	50	1250	33.5	28.2	0.84
CH_3COOH	50	870	33.5	31.9	0.95
CH_3COOH	2	35	33.5	36.8	1.10

[a] Conclusions but not data previously reported in literature reference 5.

[b] Millimoles.

Experimental data were obtained in the same fashion as when testing for amine substitutes. Twenty-five milliliters of dimethylformamide containing 33.5 millimoles of iodine and an equal volume of pyridine containing about 6 ml. of sulfur dioxide were mixed, the indicated volume of test solvent added immediately, and the resulting solution titrated with standard water in dimethylformamide as shown in Table XI.

In general, the endpoints were a bright orange somewhat more difficult to determine than the orange-yellow given by methanol.

On the assumption that the dimethylformamide might have had some effect, a similar series of tests was made on freshly prepared solutions of iodine and sulfur dioxide in pyridine to each of which 50 ml. of one of the following solvents were added: benzene, purified anhydrous dioxane, glacial acetic acid, absolute ethanol, acetonitrile, dimethylformamide, pyridine, or methanol. The solutions then were titrated with standard water in pyridine solution. Using benzene or dioxane, the water equivalence after 24 hours approached that of reaction (5), amounting to as much as 92 per cent of the theoretical value, whereas the methanolic reagent was only about 50 per cent of theory after the same time interval. However, since the endproducts of the Fischer reaction were insoluble in these materials, the resulting heavy precipitate obscured the endpoint. Furthermore, the reagent was not particularly stable in these solvents for in as little as 24 hours the solutions darkened considerably. This same behavior was noted to a lesser extent with glacial acetic acid, acetonitrile, and pyridine. In pyridine the reagent on standing often became dark green. Ethanol occasionally showed some tendency to form a precipitate and the endpoint, a deep orange, was more difficult to see than the lighter shade given by the methanol control. Dimethylformamide, while free of most of the above objections, tended toward a slightly deeper endpoint than methanol. All nonalcoholic solvents, also, made the solution subject to reactions (6) and (7) to an uncontrolled extent.

Because of their relative purity and availability, coupled with stability and ease of titration of the resulting reagent, methanol and pyridine were adopted as standard components of Karl Fischer reagent. With a few exceptions, the reporting users of this reagent have likewise employed these materials.

CHAPTER IV

Titrimetric Procedures for Using Karl Fischer Reagent

A. Recommended Preparation of Karl Fischer Reagent

To prepare 9 liter quantities of "stock" reagent, 762 grams (3.0 moles) of resublimed U.S.P. iodine are transferred to a 9 liter Pyrex glass-stoppered bottle and 2.4 liters (29.7 moles) of C.P. pyridine (<0.1 per cent water) are added. The mixture is shaken for about 10 minutes until the iodine has dissolved and 6 liters of dry synthetic methanol (preferably <0.05 per cent water) are added. (In this form the solution may be kept indefinitely since little if any loss in strength occurs before the addition of sulfur dioxide (Chapter III).[1] The homogeneous "stock" solution is stored until a few days before use, at which time a 3 liter quantity is transferred to a 4 liter interchangeable glass-stoppered buret reservoir which is cooled in a slurry of chopped ice. At the same time about 135 ml. (3 moles) of sulfur dioxide are condensed in a cold trap immersed in a carbon dioxide–methanol bath, taking care to avoid absorption of moisture from the atmosphere. The liquid sulfur dioxide is added rapidly but carefully, with shaking, to the cold iodine solution. Then the buret reservoir is stoppered and the contents allowed to return to room temperature. After the pressure has been released (by momentarily loosening the glass stopper), the solution is set aside for a day or two before being put into use. (The final reagent is about twice as strong as that employed by Karl Fischer[2] but is essentially the same in other respects.)

The recommended procedure for the preparation of the "stock" reagent saved time by making use of the faster solubility of iodine in pyridine than in methanol. However, as demonstrated in the previous chapter, the order of addition had no effect on the strength

---

[1] Smith, D. M., Bryant, W. M. D., and Mitchell, J., Jr., *J. Am. Chem. Soc.*, 61, 2407–12 (1939).

[2] Fischer, Karl, *Angew. Chem.*, 48, 394–6 (1935).

of the final reagent. Almy and his coworkers[3] suggested the use of a stock solution of sulfur dioxide in pyridine to which methanol and iodine were added a day or two before use. A similar technique was employed by Wernimont and Hopkinson.[4]

The procedures of Almy, Griffin, and Wilcox[3] and Wernimont and Hopkinson[4] were typical of other variations in the preparation of Karl Fischer reagent. The former authors prepared a "stock" solution of 203 grams (3.2 moles) of liquid sulfur dioxide in 3.785 liters (46.8 moles) of pyridine. To 1359 grams of the pyridine-sulfur dioxide solution were added 1786 grams (2.26 liters) of methanol and 453.6 grams (1.8 moles) of iodine. The final reagent was equivalent to about 5.3 milligrams of water per milliliter of reagent. The latter authors[4] dissolved 380 grams (5.94 moles) of gaseous sulfur dioxide in 700 ml. (8.9 moles) of pyridine and then added an additional 1 liter (12.7 moles) of pyridine, 200 ml. of dry methanol, and 500 grams (1.97 moles) of iodine. This reagent also was equivalent to about 5.3 milligrams of water per milliliter of reagent.

Several of the chemical supply houses offer for sale the two stable binary mixtures, iodine in methanol and sulfur dioxide in pyridine, which, when mixed, give a reagent fully as effective as that prepared in any other way.

Samples of Karl Fischer reagent sold by two laboratory supply houses were tested in the authors' laboratory. One of these was quite satisfactory but the other contained a deep red dye, making it impossible to observe the visual endpoint.

The addition of sulfur dioxide to pyridine or to the recommended "stock" reagent was accompanied by the evolution of a considerable amount of heat. Although either liquid or gaseous sulfur dioxide could be used, the former was usually preferable. Where the gas was used, the heat evolved was increased by the heat of condensation of sulfur dioxide so that more efficient cooling was necessary. Only about 15 minutes were required to collect the 135 ml. (192 grams) of liquid sulfur dioxide used for 3 liters of reagent and, since a large excess was used, the volume could be determined with sufficient accuracy in a roughly calibrated cold trap immersed in a carbon dioxide–methanol bath. To prevent undesirable overheating, Johnson[5] found that the rate of sulfur dioxide addition must be maintained at about 40 grams per hour per liter of solution initially at room temperature.

---

[3] Almy, E. G., Griffin, W. E., and Wilcox, C. S., *Ind. Eng. Chem., Anal. Ed.,* 12, 392–6 (1940).

[4] Wernimont, G., and Hopkinson, F. J., *Ibid.,* 15, 272–4 (1943).

[5] Johnson, C. M., *Ibid.,* 17, 312–6 (1945).

The recommended reagent for general use ordinarily is equivalent initially to about 3.5 milligrams of water per milliliter, using C.P. reagents. The presence of moisture in the components of the reagent obviously reduces the available iodine concentration by an equivalent amount. For this reason the water content of the solvents used should not exceed 0.1 per cent (equivalent to about 1 gram of water per liter); the presence of a few grams of water is sufficient to exhaust the reagent completely.

Karl Fischer[2] dried methanol by treatment with freshly dehydrated copper sulfate followed by careful distillation. The pyridine was allowed to stand for several hours with calcium hydride and then was distilled. Although Karl Fischer and others have recommended such treatment, preliminary dehydration of the components of the reagent generally can be avoided, since these materials usually can be purchased in sufficient purity from several sources.

Occasionally pyridine has contained excessive quantities of water. The most convenient and rapid technique for dehydrating this material was based on removal of the water as the benzene azeotrope. In a typical case, pyridine containing 1.2 per cent water was dried to less than 0.01 per cent by adding a calculated 10 per cent excess of benzene over that required to remove the water initially present and fractionating out the benzene–water azeotrope and the excess benzene.[6] Distillation of the pyridine obviously was unnecessary, since both the binary mixture and the benzene boil at much lower temperatures.

Material	B.P., °C.	Remarks
Benzene–water azeotrope	69.3	Heterogeneous; 8.9% H_2O
Benzene	80.1	
Pyridine–water azeotrope	92.3	41% H_2O
Pyridine	115–6	

Although methanol might be substantially anhydrous when purchased, it is sufficiently hygroscopic that it should be tested for moisture content before use if there has been any opportunity for absorption of moisture. When it must be dried, the alcohol may be fractionated through a 6 foot, glass-ring packed column to give a

---

[6] Unpublished results from the authors' laboratory.

distillate containing 0.02 to 0.05 per cent water.[7] Preliminary treatment with common drying agents did not seem to offer any advantages over direct fractionation.

B. Standardization of the Reagent

Although parasitic side reactions continually decrease its effective strength, the Fischer reagent is so readily standardized empirically that the lack of stability is only a minor consideration in its analytical use.

Regardless of the titrimetric method employed, the standardization involves the titration of a known weight of water with the reagent. As long as water is present, the iodine color of the reagent is discharged immediately. (The color of spent reagent is chromate yellow.) When all of the water has reacted, the iodine color persists.

If made correctly, various preparations of the Karl Fischer reagent show about the same rate of degradation. After one week, one standardization a day suffices for most routine work. For maximum accuracy, however, the water equivalence of the reagent should be checked with each set of samples.

Karl Fischer's suggested use of a methanol solution of known water content[2] has proved to be a convenient, precise, and accurate basis for the standardization; 10 ml. portions of a solution, containing about 150 milligrams of water, usually have given a reasonable titer ranging from about 40 ml. to 80 ml. of Karl Fischer reagent.

1. Preparation of Water-in-Methanol Standard

To prepare 1 liter quantities of standard, a dry 1 liter, glass-stoppered volumetric flask is filled to within about 100 ml. of the mark with dry methanol (<0.1 per cent water) and placed in a thermostatically controlled water bath (25°C. is convenient), together with a smaller dry flask containing about 200 ml. of the same methanol. About 15 grams of distilled water are weighed carefully into the liter flask and, after the contents have reached the thermostat temperature, the volume is adjusted to the mark with methanol from the smaller flask.

---

[7] Jones, G. K., *Paint Manuf.*, 15, 360–3 (1945).

2. STANDARDIZATION OF STANDARD WATER-IN-METHANOL SOLUTION AND OF KARL FISCHER REAGENT

Several 25 ml. samples of the dry methanol and at least duplicate 10 ml. portions (calibrated pipet) of that to which water has been added (water standard) are transferred to dry flasks and titrated with the unstandardized Fischer reagent. Unless the titration flasks have been stored in a desiccator, it is desirable to add a known volume of an inert solvent, such as methanol, to each flask and later to correct the titers by the quantity of reagent consumed on titration of the same volume of solvent in the same type flask.

This procedure compensates for the small amount of moisture within the flask at equilibrium with the atmosphere. Although flasks which had been dried at 110–130°C. and then immediately stored in a desiccator contained a negligible quantity of water ($<$ 0.2 milligram), those allowed to cool in the laboratory contained 0.6 milligrams (100 ml. volumetric flask), 1.2 milligrams (250 ml. volumetric flask), 1.6 milligrams (250 ml. iodine flask), 4.5 milligrams (500 ml. iodine flask), and 15.6 milligrams (1000 ml. bottle).

The net titers should check within 0.1 ml. The total water content of the standard (and, incidentally, the water equivalence of the reagent) is calculated as follows:

Let a = ml. of Fischer reagent required to titrate volume of methanol added to each flask to compensate for adsorbed moisture.

b = ml. of Fischer reagent required to titrate volume of methanol added to each flask to compensate for adsorbed moisture plus 25 ml. of methanol.

Then, $b - a = c$ = ml. of Fischer reagent required to titrate 25 ml. of methanol.

d = ml. Fischer reagent required for 9.85 ml. methanol (calc. from c, average titer of 25 ml. of methanol; for practical purposes, 10 ml. of water standard contain 9.85 ml. of methanol and 0.15 ml. of water).

e = av. ml. of reagent required for 10 ml. of water standard (corr. for adsorbed moisture on walls of flask).

f = known mg. of water added to 10 ml. of water standard.

Then, $e - d$ = ml. of Fischer reagent required for f alone and $f/(e - d) = g$ = mg. of water equivalent to 1 ml. of Fischer reagent.

Finally, $f + (g \times d)$ = mg. of water in 10 ml. of standard.

The water standard is transferred to a dry 1 liter, glass-stoppered Pyrex bottle where it may be stored for several months in a constant temperature bath at 25 ± 1°C. without any excessive change in water concentration. Two solutions in regular use, initially containing 147.6 and 156.7 milligrams of water per 10 ml., were found to contain 148.1 and 158.7 milligrams of water at the end of three and six months, respectively.

Periodic checks have been made by determining the water equivalence of a Fischer reagent simultaneously against the standard water solution and a known quantity of water.[3-5]

The stability of a water standard containing about 5 milligrams of water per milliliter of methanol solution was determined by Wernimont and Hopkinson.[4] Their results, given below, are the average of at least four individual determinations, the maximum deviation of which was 6 parts per thousand and the average deviation, about 2 parts per thousand; primary standardization of the Fischer reagent was made using weighed quantities of water.

Time after preparation of water standard, days	Mg. water/ml. standard water solution
1	5.24
12	5.23
42	5.26

Some chemists have preferred to use a regular primary standardization with a weighed quantity of water varying from 10 to 250 milligrams.[3,4,8-13]

The reagent has been standardized with an ethanol solution containing 5 to 8 per cent water, based on accurate density measurements.[14] Recently the use of stable hydrates has been recommended, including ammonium oxalate,[12] $(COONH_4)_2 \cdot H_2O$; sodium acetate,[15] $CH_3COONa \cdot 3H_2O$; citric acid,[7,16] $C_6H_8O_7 \cdot H_2O$; potassium aluminum sulfate,[7] $K_2Al_2(SO_4)_4 \cdot 24H_2O$, and ferrous am-

---

[8] Zavarov, G. V., *Zavodskaya Lab.*, **5**, 1314–8 (1936).

[9] *Am. Soc. Testing Materials, Standards*, II, 1535 (1944).

[10] Fosnot, R. H., and Haman, R. W., *Cereal Chem.*, **22**, 41–9 (1945).

[11] Seaman, W., Norton, A. R., Woods, J. T., and Massad, E. A., *Ind. Eng. Chem., Anal. Ed.*, **16**, 517–9 (1944).

[12] Rennie, R. P., and Monkman, J. L., *Can. Chem. Process Inds.*, **1945**, 366–70.

[13] Kaufmann, H. P., and Funke, S., *Fette u. Seifen*, **44**, 345–69 (1937).

[14] Levy, G. B., Murtaugh, J. J., and Rosenblatt, M., *Ind. Eng. Chem., Anal. Ed.*, **17**, 193–5 (1945).

[15] Warren, G. G., *Can. Chem. Process Inds.*, **1945**, 370.

[16] Cornish, G. R., *Plastics*, **1946**, 99–103.

monium sulfate,[7] $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$. Sodium acetate trihydrate was chosen by Warren[15] after an extensive study of likely solid standards. The ordinary reagent grade hydrated salt was reported to be pure, nonhygroscopic, and extremely soluble in alcohol and in the Fischer reagent. Citric acid monohydrate, however, needed to be specially prepared, because of its natural hygroscospicity, by desiccation over dehydrated citric acid.[7]

C. Macrotitrimetric Methods

1. Visual Endpoint

a. Apparatus

Karl Fischer reagent is extremely sensitive and, if permitted to come into prolonged contact with atmospheric moisture, will absorb and react with sufficient water to introduce appreciable errors in any determination. It is essential, therefore, that the reagent in the buret and reservoir be adequately protected against contact with moist air. The standard laboratory desiccants, calcium chloride and calcium sulfate, are satisfactory for this purpose. Either automatic burets of the types shown in Figures 5 and 6 or the bottom filling unit of Figure 7 are convenient. In both types the construction is completely of Pyrex. The buret reservoirs, connected to the burets with standard taper grinds, are of 4 liter capacity.* The buret tips are enlarged to about 1 mm. inside diameter. Although either suction or pressure can be used to fill burets of this type, the former method is preferred. Vacuum filling permits a gradual steady introduction of air through the desiccant-protected inlet, instead of the sudden rush of air usually experienced when pressure filling is used.

The pronounced sensitivity of the reagent was pointed out by Fischer[2]: "The reaction is so sensitive that a sample which has been titrated to a distinct brown endpoint changes immediately to yellow if one breathes gently toward the flask. Therefore, a better indicator is unnecessary." It is essential that titration flasks offering a minimum of exposure to the atmosphere be used. In place of the open Erlenmeyer flasks used by some investigators, volumetric flasks are recommended. During titration the elongated narrow neck of this type of flask offers the buret tip complete protection and at

---

* These burets were made by Eck and Krebs, 131 West 24 Street, New York, N. Y.

the same time permits continuous agitation. During titration, care should be taken to avoid excessive exposure of the buret tip to the atmosphere (by removing the flask); otherwise measurable amounts of water may be absorbed. The sample, for which water is to be determined, may be weighed or pipetted into a 100 ml. or 250 ml. dry glass-stoppered volumetric flask (see Figs. 5 and 7).

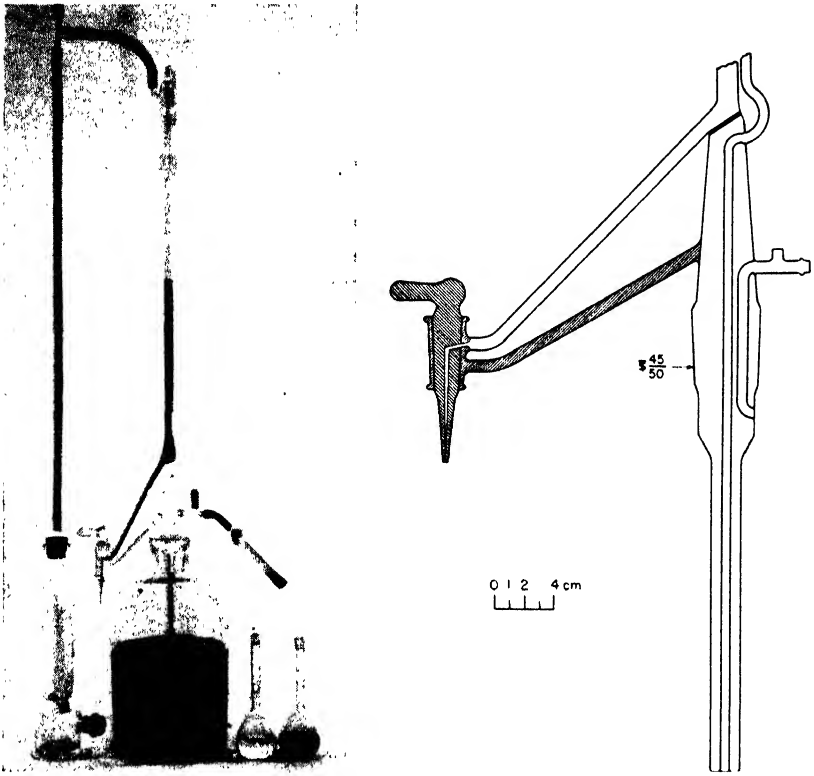


Fig. 5. Automatic 50-ml. buret for visual titrations.[6]

Volumetric flasks are dried with difficulty. After washing with water, acetone, or alcohol, several hours' drying in a static oven at 110–130°C. is required. It is convenient to allow flasks to dry overnight before use. Drying time probably could be reduced by employing forced air circulation.

Where a completely closed system is desired (in the titration of small quantities of water), a unit of the type shown in Figure 6 may be used. The flask is a 250 ml. Erlenmeyer to which a standard 28/15 spherical grind (outer member) has been sealed. The corresponding inner member is connected to the base of the buret stopcock in such a way that the buret tip extends just beyond the grind; a vent permits attaching a drying tube which in turn may be supported from the glass rod used to strengthen the extended stopcock.

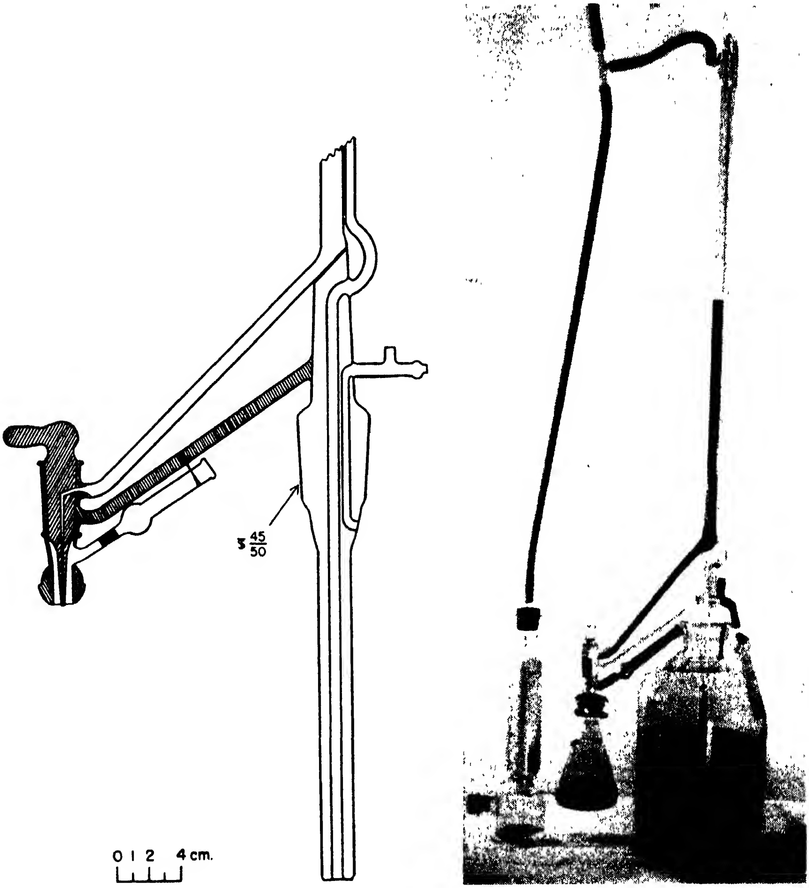


Fig. 6. Automatic 50-ml. buret for visual titrations in a closed system.[6]

Sufficient freedom is given by the spring type clamp to allow continuous agitation of the contents of the flask during titration.

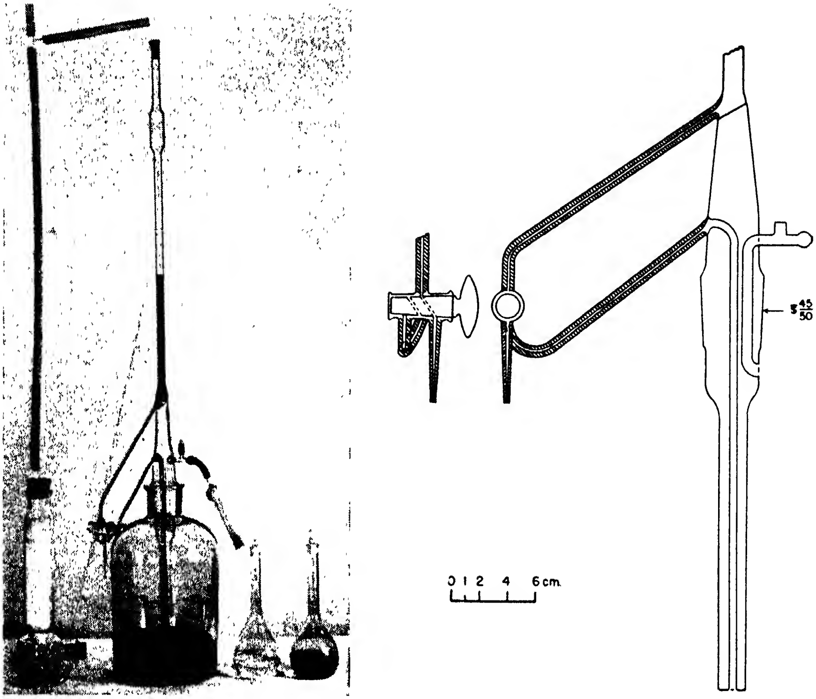


Fig. 7. Bottom-filling 100-ml. buret.[6]

b. Titration

The sample, usually containing from 50 to 250 milligrams of water, is weighed or pipetted into the titration flask. A known volume of a relatively dry inert solvent or dispersing agent, e.g., methanol, is added and the mixture titrated directly with Karl Fischer reagent delivered from the protected buret. As the endpoint is approached, as evidenced by a decrease in the rate of discharge of the brown color and a slight change in shade of "spent" reagent from canary to chromate yellow, the reagent is added in 0.1 to 0.2 ml. increments until the brownish color of iodine persists. The endpoint change from chromate yellow to the red-brown of iodine is sharp and reproducible. After correction for water in the solvent, the net titer is equivalent to moisture in the sample. In some cases it is desirable—particularly where small quantities of water are involved—to "pretitrate" the solvent before addition of the sample. The subsequent titer then represents only water in the sample being analyzed. For routine purposes this latter technique has the obvious disadvantage of requiring a double titration on every sample.

The visual endpoint is easily mastered; routine operators have been trained within a few hours. As a guide it has been desirable to maintain double standards for comparison, one showing the yellow color of "spent" reagent and the other, the iodine endpoint. These standards are prepared readily from duplicate standardizations of the reagent. After titration of the contents of the first flask to the brown endpoint, a small quantity of wet methanol is added, sufficient to produce the discharged color. The second flask is titrated to the endpoint and is tightly stoppered.

(An arbitrary standard of $N/300$ iodine solution has been recommended[7]; this is obviously unnecessary since the above standards are completely satisfactory.)

This same technique can be applied to samples which are colored sufficiently to obscure the normal endpoint. For the first titration of a material of this type, no attempt is made to choose the endpoint accurately. Instead, the reagent is added until a definite color change is observed. After the addition of a drop of water which gives the color of spent reagent in addition to that imparted by the sample, one is able subsequently to determine fairly accurately the water content of additional samples, using the "discharged color" as a guide.

The maximum sensitivity of the visual titration is obtained only on clear, slightly colored liquids. In some cases it is practical to use a clear, water-miscible liquid for the extraction of small quantities of water from dark solutions in which the extracting liquid is insoluble.

Milberger and his coworkers[17] have recommended a visual back titration method. Excess Fischer reagent is added to the sample,

---

[17] Milberger, E. C., Uhrig, K., Becker, H. C., and Levin, H., paper presented before the Analytical Section, 110th meeting of the American Chemical Society, Chicago, Ill., September 10, 1946.

after which the solution is back titrated with standard water-in-methanol, equivalent to about 2.5 milligrams of water per milliliter, to the chromate yellow color of discharged reagent. Although satisfactory, this technique offers no advantages over the direct titration and requires an additional buret to deliver the standard water-in-methanol solution.

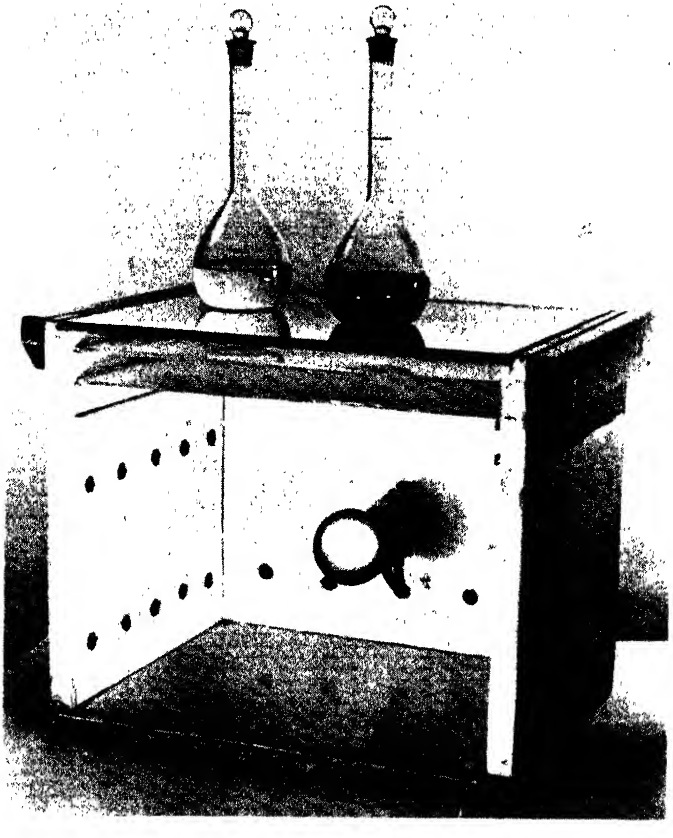


Fig. 8. Daylight lamp, vertical illumination, with front removed to show positioning of bulb and frosted glass plates.[6]

As an aid in determining the endpoint, special lighting is often desirable. Daylight lamps permitting vertical or horizontal illumination, such as those shown in Figures 8 and 9, are suitable for this

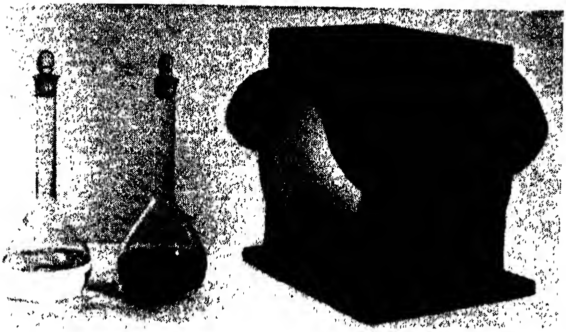


Fig. 9. Daylight lamp, horizontal illumination.[6]

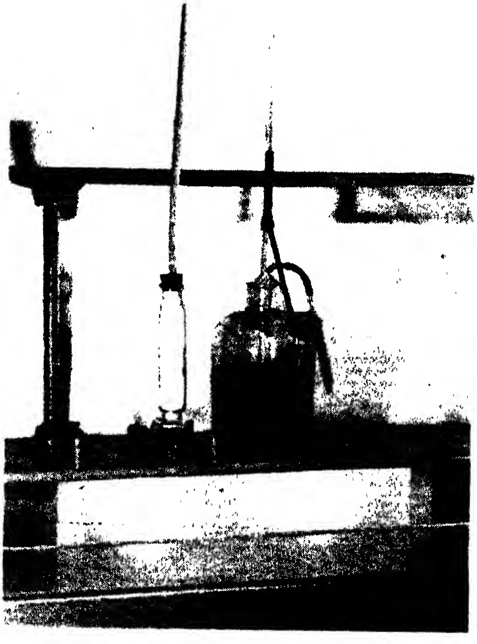


Fig. 10. Titration table combining vertical and horizontal illumination.[6]

purpose. Both types may be incorporated directly into a titration table, such as that shown in Figure 10, which presents the added advantage of having the light source directly under the sample during titration. One of the most sensitive methods depends on horizontal observation through the solution held at eye level, using normal daylight as a background, although this procedure requires removal of the flask from the buret tip when the endpoint is approached closely.

The visual endpoint has had the particular advantage of permitting rapid titrations requiring only simple apparatus. In general, macrotitrations have been duplicated on a routine basis with a maximum variation of 0.2 ml., equivalent to about 0.5 milligram of water. With a sample size chosen to contain 125–200 milligrams of water, the precision and accuracy approached 0.1 to 0.2 per cent for samples run in duplicate. For smaller amounts of water, more care was required. The visual titration of several milligrams of water could be reproduced to ±0.1 ml. of the average volume of Fischer reagent, provided that the usual precautions of good analytical technique were observed. This is demonstrated by the data in Table XII, in which several different volumes of dry methanol were titrated in duplicate, using 100 ml. glass-stoppered volumetric flasks for volumes up to 50 ml., and 250 ml. flasks for the remaining samples.[6]

TABLE XII

Determination of Water in Dry Methanol[6]

Sample volume, ml.	Fischer reagent, ml.[a]	Ml. reagent per 10 ml. methanol	Water found	
			Mg.	Wt. %
10	0.30 ± 0.00	0.30	0.8	0.0095
25	0.65 0.05	0.26	1.7	0.0085
50	1.25 0.10	0.25	3.3	0.008
100	2.45 0.05	0.25	6.45	0.008
200	5.00 0.10	0.25	13.2	0.0085

[a] Titer corrected for blank on 25 ml. of methanol by titration of an equal volume in the same type flask.

A comparison with the acetyl chloride hydrolysis procedure of Smith and Bryant[18] demonstrates that for small quantities of water the precision favors the Fischer method. In the data in Table XIII duplicate 50 ml. quantities of benzene and purified dioxane were used for each analysis.[6]

---

[18] Smith, D. M., Bryant, W. M. D., *J. Am. Chem. Soc.*, **57**, 841–5 (1935).

TABLE XIII

Analyses for Water by Karl Fischer and Acetyl Chloride Procedures[6]

Substance	Water found, wt. %	
	Fischer titration	Acetyl chloride method
Benzene	0.0285 ± 0.0005	0.027 ± 0.001
Dioxane	0.004 0.000	0.003 0.001

2. Electrometric Endpoints

a. Titration Assemblies

The various electrometric methods for the determination of the Fischer reagent endpoint have employed the same type of titration assembly. Almy, Griffin, and Wilcox[3] used a standard 150 ml. extraction flask fitted with a Bakelite adapter which was tapered at the base but which had a cylindrical extension to permit clamping (Fig. 11). Holes were drilled in the adapter to just permit passage

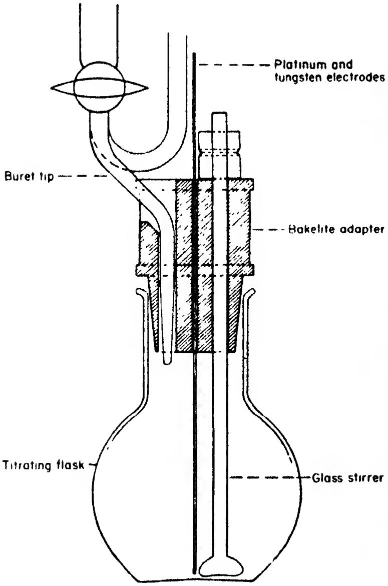


Fig. 11. Titration assembly for electrometric endpoint.[3]

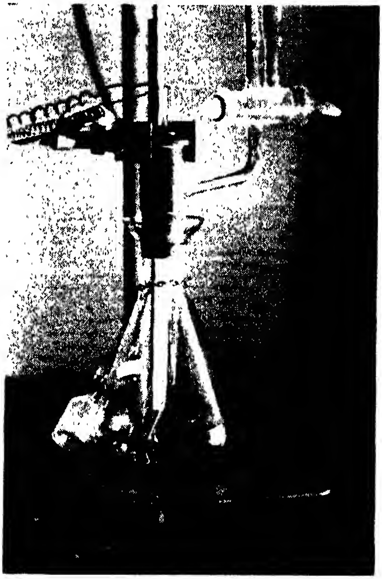


Fig. 12. Titration assembly.[4]

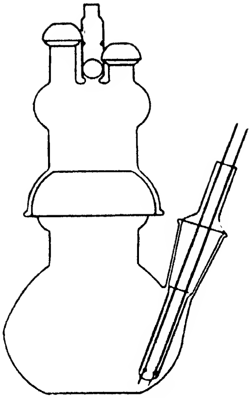


Fig. 13. Titration flask for electrometric endpoint.[23]

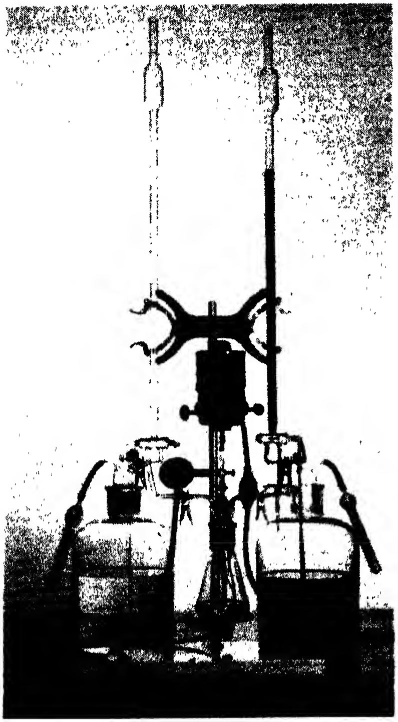


Fig. 14. Titration assembly.[6]

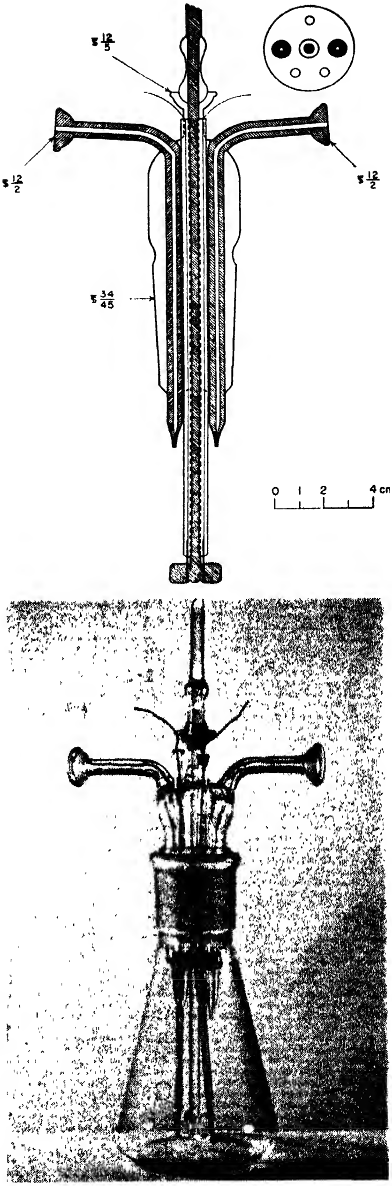


Fig. 15. Titration flask for assembly in Figure 14.[6]

of the electrodes, buret tip, and the glass stirrer controlled by a variable speed motor. The buret, fitted with a three-way stopcock, was connected to a reagent reservoir suitably protected against outside moisture. The buret tip extended into the flask slightly below the adapter to prevent wetting of the Bakelite during addition of reagent.

The titration apparatus of Wernimont and Hopkinson[4] (shown in Fig. 12), similar to that of Almy, Griffin, and Wilcox, consisted of a Bakelite adapter turned to fit the grind of a 250 ml. iodine flask and drilled to permit passage of a stainless steel stirrer, buret tip, and electrodes. Approximately the same type of unit has been used by several other investigators.[16,19–21] Cornish[16] employed carbon dioxide as stirring agent. Kieselbach[22] recommended an all glass modification of the Wernimont-Hopkinson unit (Fig. 12), involving a standard 24/40 flask grind, glass stirrer, and standard 10/30 buret connection. Zerban and Sattler[23] used a specially designed flask with a 65/40 ball joint (Fig. 13). In this case the upper, small (18/9) spherical grinds fitted directly into outer members sealed on the buret stopcocks, the tips of which were 16.5 cm. long. A standard 14/35 side tube accommodated the electrodes, while a check valve acted as a vent whenever liquid was being admitted into the flask from the burets. Magnetic stirring was employed.

The electrometric apparatus used in the authors' laboratory[6] for those cases when the visual endpoint was impractical is illustrated in Figure 14. Although the principle was the same as that of other examples of equipment of this type, the connections were all Pyrex. Seals between burets and reservoirs and burets and titration assembly were made through standard 12/2 spherical grinds. The titration assembly, shown in Figure 15, consisted of a standard 34/45 grind, 250 ml. Erlenmeyer flask fitted with an adapter containing two buret inlets, glass stirrer, "breathing tube," and sealed-in electrodes. The stirrer was made from a standard 12/5 spherical grind which permitted variable speed, smooth stirring.

---

[19] McKinney, C. D., Jr., and Hall, R. T., *Ind. Eng. Chem., Anal. Ed.*, **15**, 460–2 (1943).

[20] Heinemann, B., *J. Dairy Sci.*, **28**, 845–51 (1945).

[21] Carter, R. J., and Williamson, L., *Analyst*, **70**, 369–71 (1945).

[22] Kieselbach, R., *Ind. Eng. Chem., Anal. Ed.*, **18**, 726–7 (1946).

[23] Zerban, F. W., and Sattler, L., *Ibid.*, **18**, 138–9 (1946).

The outer grind of this stirrer was sealed to the flask. The inner grind had a long shaft through it (ring sealed above the grind), which carried the stirrer blades on the lower end and was connected to the variable speed motor at the upper end. When lubricated with a thin film of stopcock grease, the spherical grind formed an airtight seal under slight positive thrust from the stirring motor.

When necessary, the "breathing tube" could be protected with a drying tube. The adapter was elongated so that it could be clamped readily.

In those cases where a volumetric flask was desirable—for example, titration for water after a reaction at elevated temperatures—the apparatus shown in Figure 16 was satisfactory.[6] The adapter for this purpose contained a single buret tip extension. Magnetic stirring was used to insure continuous mixing.

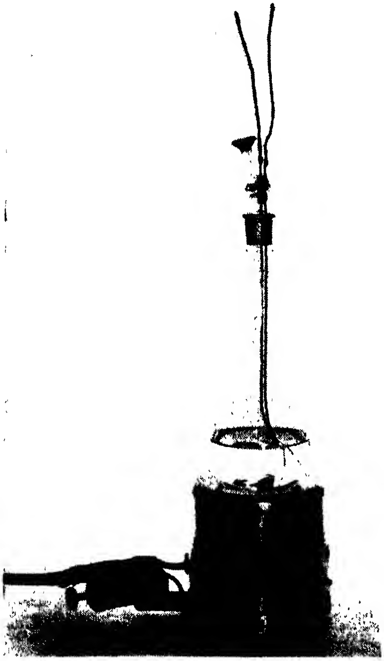


Fig. 16. Volumetric flask for electrometric titrations.[6]

b. Potentiometric Back Titration Method

In the early work on electrometric determinations of the Karl Fischer endpoint the conditions chosen were usually such that "back titration" techniques gave more sensitive and accurate endpoints. Further work, however, disclosed that direct titrations could be made with equal sensitivity and accuracy, using substantially the same apparatus but under somewhat different conditions. These direct titrations are discussed later in this chapter.

The earliest reported Karl Fischer titration employing an electrometric principle was described by Almy, Griffin, and Wilcox,[3] who successfully employed a potentiometric technique for the detection of the endpoint. In addition to being generally applicable to dark colored solutions, the potentiometric method was found to increase the sensitivity of the reagent.

Karl Fischer was the first to suggest that an electrical titration process might be applied practically to the analysis of liquids for water. The possibility of employing bimetallic electrode systems in potentiometric titrations was first suggested by Hostetter and Roberts,[24] who proposed that, in the titration of ferrous ion, palladium might be substituted for the calomel electrode, platinum serving as the indicator electrode. This idea first was applied practically by Willard and Fenwick[25] who used electrodes of dissimilar "inert" metals, including the platinum–tungsten system, for the titration of ferrous ions. They also included in their study the behavior of two platinum electrodes; in this case an initial potential break was attributed to a strained condition in the freshly sealed junction of metal to soft glass, since subsequent titrations failed to give an endpoint. They concluded that, since successive titrations dissipated the strain, the endpoint, where bimetallic systems were employed, was a polarization phenomenon.

In a second simultaneous publication, Willard and Fenwick[26] demonstrated empirically that the action of these electrodes depended on dissolved gas reactions taking place on their surfaces; a metallic electrode functioned as a gas electrode and the potential thus fixed by the magnitude of the solution pressure of the gas adsorbed on the electrode. In systems using two "inert" metallic electrodes, the endpoint indication for oxidation-reduction reactions was dependent on the difference in solvent power of the two metals for gas.

In the development of their method, Almy and co-workers[3] found that a small but definite change in potential occurred when a platinum–tungsten electrode pair was permitted to contact the Fischer reagent during final discharge of the iodine color by a standard water-in-methanol reagent, the tungsten becoming more positive with respect to platinum.

The tungsten electrode must be cleaned and sensitized by dipping the ends of the wire for 5 to 10 seconds in fused sodium nitrite at slightly above the melting point of the salt,[27] finally washing with water, and drying by rinsing with acetone or methanol. This process was repeated whenever the wire became tarnished.

A technique using standard water-in-methanol reagent to back titrate excess Fischer reagent was found to give a sharper and more reproducible endpoint than that given by direct titration of the sample with Fischer reagent as employed in the visual procedure.[3]

---

[24] Hostetter, J. C., and Roberts, H. S., *J. Am. Chem. Soc.*, 41, 1337–57 (1919).

[25] Willard, H. H., and Fenwick, F., *Ibid.*, 44, 2504–15 (1922).

[26] Willard, H. H., and Fenwick, F., *Ibid.*, 44, 2516–29 (1922).

[27] Smith, G. F., and Sullivan, V. R., *Electron Beam Sectrometer,* 1st ed., G. Frederick Smith Chemical Co., Columbus, 1936, p. 39.

The magnitude of the potential increase is shown in Figure 17, based on the measurement of apparent *p*H (converted to millivolts) during the slow addition of methanol containing 6.09 milligrams of water per milliliter, in increments of 0.1 ml., to 30.20 ml. of Fischer reagent. The time element was important since more than 10 seconds was required for the full potential to develop. With a sensitive *p*H meter about half of this change developed in the first second. The precision of the endpoint was determined by the titration of 32.10 ml. of Fischer reagent (Fig. 18). As indicated in the figure, the water-in-methanol reagent was added dropwise after 25.5 ml. had been added. A sharp potential change was exhibited between drops 173 and 174. At a delivery of 1 drop per second, the endpoint could be determined to within 0.03 ml. from an average buret tip while, at about 5 drops per second, the endpoint might be overrun by as much as 0.1 ml., which in this case was equivalent to about 0.6 milligram of water.

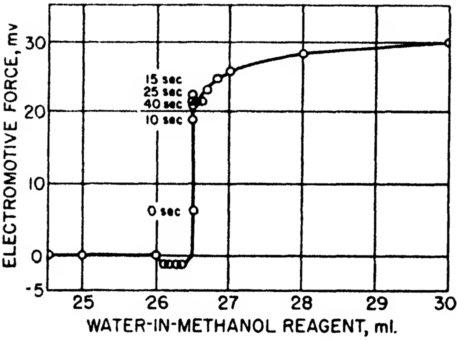


Fig. 17. Change in potential during titration of Fischer reagent with water-in-methanol.[8]

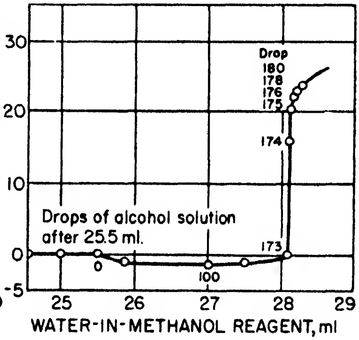


Fig. 18. Precision of potentiometric titration of Karl Fischer reagent with water-in-methanol.[8]

A titration of this type required a sensitive millivoltmeter capable of immediately registering a potential change and exhibiting a strong positive deflection for the total increase of 20 millivolts at the endpoint. No current could be drawn from the electrodes, since, under these conditions, they tended to polarize markedly. The authors[8] indicated that any vacuum tube millivoltmeter could be used which incorporated high sensitivity (at least half the full deflection for an applied 20 millivolts) and current stability without drawing measurable current from the cell. (Any research model *p*H meter would be satisfactory.) Numerical values were unimportant, provided the incidence of potential change was marked.

Before carrying out an analysis the titration assembly is clamped into position. The platinum and tungsten electrodes, connected through copper leads, are attached directly to the input terminals of a sensitive *p*H meter. The sample, containing 0.05 to 0.2 gram of water, is transferred to the flask. Karl Fischer reagent from an appropriate protected buret assembly is added to excess, the flask is connected to the titration assembly, and the water-in-methanol buret tip is introduced through the adapter (see Fig. 11). The meter is adjusted so that its needle is at the midpoint of the scale. With the stirrer running at a sufficient speed to insure quick mixing as the titration proceeds, the water-in-methanol solution, containing 5 to 6 milligrams of water per milliliter, is added rapidly until—for light colored samples—the color starts to change from brown to orange. At this point the meter key is locked down and the alcohol solution is added dropwise. At about 1 milliliter short of the endpoint, the needle is deflected slightly and then, as the titration continues, is completely displaced in the opposite direction across the midpoint and off the scale. The titration is terminated when this occurs. If this deflection is caused by a temporary local concentration of water at the electrodes, the needle will return, requiring the further addi-

TABLE XIV

Standardization of Fischer Reagent by Potentiometric Method[3]

Water taken, g.	Iodine reagent, ml. (*A*)	Alcohol solution, ml. (*B*)	*A/B* (*R*)	Water equivalence factors, mg./ml.	
				Iodine solution	Alcohol solution
	27.70	32.42	0.854		
	24.10	28.32	0.851		
	24.47	28.82	0.849		
	24.45	28.80	0.849		
			Average 0.850		
0.2017	40.29	2.47		5.29	4.50
0.2247	43.60	1.30		5.29	4.50

tion of a few drops of the alcohol solution. If the displacement is permanent, the endpoint has been reached.

The speed of titration thus is somewhat dependent on visual observation of the color change. Dark samples require a slower and more careful back titration. Typical standardization data, based on titration of a known weight of water, are given in Table XIV.

The equivalence ratio, *R*, must be determined daily. Since the water content of the alcohol solution remains relatively constant, the strength of the Fischer reagent can be calculated readily.

c. Dead-Stop Back Titration Method

In applying the "dead-stop"* technique to the Karl Fischer titration for water, Wernimont and Hopkinson[4] demonstrated that the method "gives a sharp and reproducible endpoint in these nonaqueous solutions with fewer manipulation difficulties than the potentiometric method and better precision than the visual method." This technique has become increasingly popular and at present is probably the most widely used electrometric method for the determination of the Karl Fischer endpoint.

In their study of bimetallic systems, Willard and Fenwick[26] found that by employing a polarizing current the platinum–platinum electrode system gave a sharper endpoint than one with a single metallic electrode or a bimetallic system of dissimilar metals. This was further demonstrated by Van Name and Fenwick[29] who found that, for a polarized platinum–platinum system, to which a constant potential of 0.2 to 0.5 volt was applied, the difference in potential in ferrous sulfate solution decreased rapidly on the addition of potassium permanganate. When the oxidation was nearly complete, the difference increased and rose sharply as the last traces of ferrous ion were oxidized, decreasing again on further addition of the permanganate. This phenomenon was explained as follows:

During the titration of ferrous sulfate with potassium permanganate the solution contains appreciable concentrations of both ferric and ferrous ions. Since the reversibility of the ferric–ferrous system is relatively high, both anodic and cathodic polarizations are nearly destroyed. Consequently, the electrodes show little or no potential difference. When the titration is nearly complete, the ferrous ion disappears while the permanganate ion appears, resulting in a

---

* The term "dead-stop" was suggested by Foulk and Bawden[28] as aptly descriptive of the characteristic action of the galvanometer in coming to rest. They felt that "polarization endpoint" might be a better name but would lead to confusion with other polarization techniques in bimetallic systems.

[28] Foulk, C. W., and Bawden, A. T., *J. Am. Chem. Soc.*, **48**, 2045–51 (1926).
[29] Van Name, R. G., and Fenwick, F., *Ibid.*, **47**, 9–18, 19–29 (1925).

sharp rise in electrode potential. The depolarizing action of ferric ion then becomes negligible, so that at the endpoint neither electrode is effectively depolarized. As the concentration of permanganate ion increases, however, cathodic depolarization becomes effective. At the resulting higher potential the manganous ion is capable of anodic depolarization. This action, therefore, tends to balance the potentials again after the endpoint has been exceeded (Fig. 19).

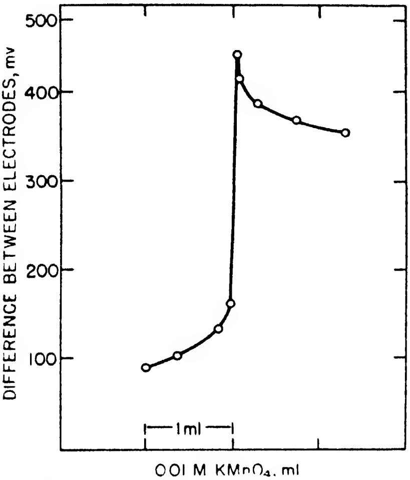


Fig. 19. Titration of 0.08 M $FeSO_4$ with $KMnO_4$ with polarized platinum–platinum electrodes.[29]

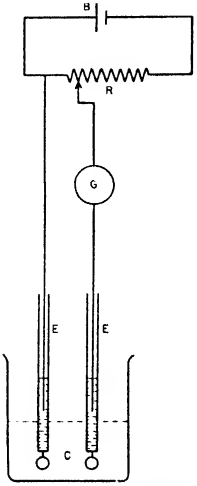


Fig. 20. Wiring diagram for dead-stop apparatus.[4,28]

The "dead-stop" endpoint, a special case of bimetallic electrode systems, was accidently discovered by Foulk and Bawden[28] while preparing for the electrometric titration of iodine in the usual manner. The high sensitivity of the endpoint and simple apparatus requirements led them to explore and develop fully this new electrometric technique. The equipment necessary for this procedure is illustrated in Figure 20, in which B is a battery, R is a rheostat, G is a galvanometer, C is the titrating vessel, and E, E are platinum wire electrodes. The fundamental requirement of the "dead-stop" technique as opposed to other methods employing bimetallic systems is the low potential difference between the electrodes. This low potential is balanced by the back electromotive force of polarization. Consequently no current flows and the galvanometer registers zero. Under these conditions the "dead-stop" endpoint is applicable to all systems in which a sharp transition from the polarization (or depolarization) of at least one electrode to the complete depolarization (or polarization) of both coincides with the end of the reaction. Foulk and Bawden assumed that "polarization is due to oxygen absorbed on the anode and hydrogen on the cathode and that the anode can, therefore, be depolarized by a suitable reducing agent and the cathode by a suitable oxidizing agent.

Three conditions can function in this technique:[28]

(*1*) One electrode is polarized. In the titration of thiosulfate or arsenite with iodine the cathode is polarized and the anode remains depolarized by the action of the reducing agents present. At the endpoint the first trace of iodine in excess depolarizes the cathode.

(*2*) Both electrodes are depolarized. This requires a solution of both an oxidizing and a reducing agent which either do not react with one another or react reversibly. An example of this case is the titration of iodine with thiosulfate, where at the endpoint the slight excess of reducing agent polarizes the cathode.

(*3*) Both electrodes are polarized. In this case the solution at the endpoint must be capable of depolarizing both, as, for example, by the use of mixed reagents. Thus a strong base may be titrated with a strong acid under these conditions, provided that small amounts of iodide ion and oxidizing agent are present in the base. By the addition of a suitable reagent condition *3* can be transformed into condition *1*.

This sensitive endpoint is characterized by a gradual shift in the galvanometer indicator as the endpoint is approached, which either stops permanently at the endpoint even though excess reagent is added (condition *2*) or is permanently displaced from a position of rest (conditions *1* and *3*).

Many oxidation-reduction titrations have been made successfully by this procedure. The discoverers applied it to titration of iodine with sodium thiosulfate or sodium arsenite (and *vice versa*), to oxalate-permanganate titrations, and to direct titration of thiosulfate with permanganate (or *vice versa*). Other applications included the determination of cuprous ion,[30] the standardization of sodium thiosulfate against potassium iodide,[31] the determination of traces of carbon disulfide employing iodometric titration of ethyl xanthate,[32] and the microtitration of selenium.[33] In this last case Wernimont and Hopkinson prepared the electrodes by sealing small loops of platinum wire into soft glass tubing. Special care was necessary since microscopic cracks around the seals vitiated the technique (Fig. 20).

In this method two clean platinum electrodes are used. In preparing the electrodes short lengths of platinum wire may be spot-welded to tungsten, the latter metal permitting a tight strain-free seal through the Pyrex sleeve.[6] Copper wire leads are used to connect to the titrimeter circuit. The platinum electrodes may be cleaned by immersion in hot sulfuric acid–dichromate solution. Otherwise, the titration assembly is arranged and the sample prepared in a manner similar to that used in the potentiometric

---

[30] Pring, M. E., and Spencer, J. F., *Analyst*, **55**, 375–82 (1930).

[31] Pagel, H. A., and Miers, J. C., *Ind. Eng. Chem., Anal. Ed.*, **10**, 334–5 (1938).

[32] Bishop, R. L., and Wallace, E. L., *Ibid.*, **17**, 563–4 (1945).

[33] Wernimont, G., and Hopkinson, F. J., *Ibid.*, **12**, 308–10 (1940).

method, except that the copper wire leads are connected through suitable resistances to the galvanometer (Fig. 20). When the resistance, *R*, is adjusted so that the potential difference between platinum electrodes immersed in active Fischer reagent is 10 to 15 millivolts, sufficient current flows through the solution to deflect the galvanometer light off the scale. During the addition of standard water-in-methanol, the indicator remains displaced until the endpoint is approached. When nearly all of the iodine is reduced, the galvanometer is affected by each addition of water solution and at the endpoint the galvanometer suddenly comes to rest at the zero point. As observed in the potentiometric method, the direct titration of the sample with Fischer reagent was found to be less sensitive.

The recommended technique is another example of the condition in which both electrodes remain depolarized during the titration (see page 88). Active Fischer reagent (which always contains iodide ion) provides the iodine-iodide couple of oxidizing and reducing agents. At the endpoint when all of the iodine has been reduced the iodide ion is then capable of polarizing the cathode after which current ceases to flow and the galvanometer returns to zero.

Exact measurement of the potential is unnecessary. Adjustment of the resistance so that the galvanometer is deflected to just off the scale with both electrodes depolarized is sufficient.

The precision and accuracy of determining water by this technique were found by titration of known quantities of water in methanol.[4] In one case weighed quantities of water were added to titration flasks containing 10 ml. portions of methanol. Excess Fischer reagent was added (determined by the permanent appearance of the iodine color) and the solution was back titrated with standard water-in-methanol to a "dead-stop" endpoint. The results are shown in Table XV.

In another set of experiments,[4] known quantities of water were diluted with dry methanol to exactly 100 ml. Various portions of this solution were transferred to the titration flasks and analyzed as above. The results are given in Table XVI.

The results in the above tables indicate a high order of precision and accuracy on quantities of water ranging from less than 4 milligrams to almost 200 milligrams.

TABLE XV

Analyses for Water in Presence of Methanol by "Dead-Stop" Endpoint Method[4]

No.	Fischer reagent added, ml.	Standard water soln. back titrated, ml.	Standard water soln. net,[a] ml.	Water, mg.		
				Found	Present[b]	Difference
1	5.00	6.20	0.70	3.7	3.7	0.0
2	5.00	6.20	0.70	3.7	3.7	0.0
3	8.00	6.60	4.43	23.7	23.3	+0.4
4	8.00	6.40	4.63	24.7	24.4	+0.3
5	10.00	4.55	9.24	49.3	50.1	−0.8
6	15.00	8.30	12.38	66.1	65.5	+0.6
7	15.00	6.85	13.83	73.9	74.0	−0.1
8	20.00	9.05	18.53	99.0	99.1	−0.1
9	20.00	4.20	23.38	124.8	125.6	−0.8
10	25.00	7.55	26.93	143.8	144.3	−0.5
11	25.00	6.00	28.48	152.1	152.5	−0.4
12	30.00	5.70	35.67	190.5	191.6	−1.1

[a] (Ml. Fischer reagent × R) − (ml. standard water solution back titrated). $R = 1.379$ ml. of standard water solution per ml. of Fischer reagent. The standard water solution contained 5.34 mg. of water per ml.

[b] Sum of water added to and found in 10 ml. of methanol.

TABLE XVI

Analyses of Known Solutions of Water in Methanol by "Dead-Stop" Endpoint Method[4]

Aliquot weight, g.	Fischer reagent added, ml.	Standard water soln. back titrated, ml.	Standard water soln. net,[a] ml.	Water, %	
				Found	Present
19.8	10.00	6.90	2.29	0.06	0.06
19.8	10.00	6.80	2.39	0.06	0.06
19.8	15.00	5.45	8.33	0.22	0.22
19.8	15.00	5.45	8.33	0.22	0.22
7.92	15.00	5.80	7.98	0.53	0.525
7.92	15.00	5.75	8.03	0.53	0.525
1.580	20.00	2.90	15.48	5.13	5.02
1.575	20.00	2.85	15.53	5.17	5.02

[a] (Ml. Fischer reagent × R) − (ml. standard water solution back titrated). $R = 0.919$ ml. of standard water solution per ml. of Fischer reagent. The standard water solution contained 5.24 mg. of water per ml.

McKinney and Hall[19] introduced a simplified "dead-stop" apparatus employing a cathode ray "magic eye" tube in place of the galvanometer and a Serfass titrimeter circuit[34] modified and

---

[34] Serfass, E. J., *Ind. Eng. Chem., Anal. Ed.,* **12,** 536–9 (1940).

adapted to the Karl Fischer endpoint (Fig. 21). The titration flask accommodating the buret, glass stirrer, and two platinum electrodes is connected through leads to the titrimeter. A small polarizing voltage is applied to the electrodes, controlled and amplified through the 6F5 vacuum tube. The amplifier is coupled to the 6E5 cathode ray tube which serves as the indicator. Power for the unit is furnished by 110 volt alternating current through a 6H6 tube acting as a rectifier-doubler.

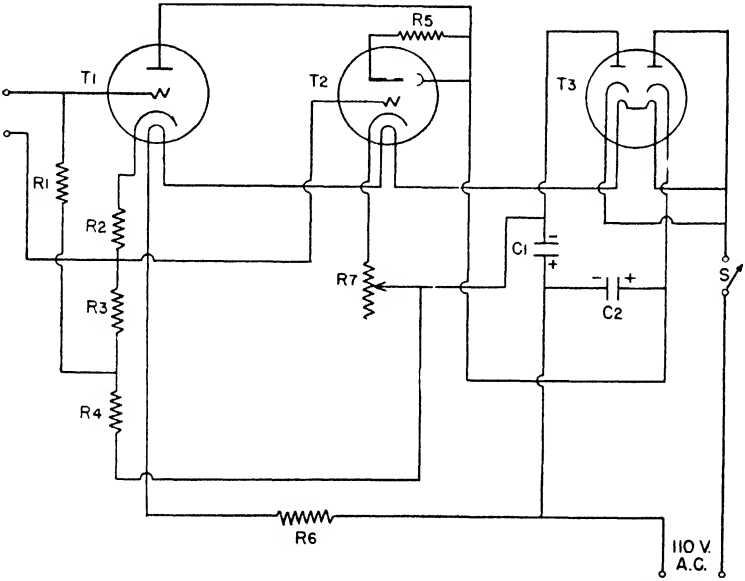


Fig. 21. Wiring diagram for electrometric apparatus.[19]

R_1,	100,000-ohm fixed resistor, 0.5 watt
R_2, R_3,	1000-ohm fixed resistors, 1 watt
R_4,	25,000-ohm fixed resistor, 1 watt
R_5,	500,000-ohm fixed resistor, 1 watt
R_6,	350-ohm resistor (in line cord)
R_7,	25,000-ohm volume control

T_1, 6F5 tube
T_2, 6E5 tube
T_3, 6H6 tube
S, single-pole, single-throw toggle switch
C_1, C_2, 4-μF electrical condenser, 450 volts

The original Serfass circuit[34] did not use the rectifier as a doubler, permitting use of both alternating and direct current supply lines. Although it cannot be employed on direct current, the present circuit gives approximately a 250 volt direct current supply for the operation of the amplifier and cathode ray tubes and has the advantage of giving a much brighter fluorescence on the target of the 6E5 cathode ray tube.

At the beginning of a titration the polarizing voltage was less than 1 millivolt, increasing to about 15 millivolts at the endpoint. The current flowing between the electrodes during the titration was about 5 microamperes, becoming practically zero at the endpoint. The initial voltage differed from that proposed by Wernimont and Hopkinson[4] who applied a potential of about 10 millivolts. The current relationships, however, remained at about the same order of magnitude.

In making a titration the sample, containing 50 to 150 milligrams of water, is transferred to the titration flask. The Karl Fischer reagent is added in 1 to 2 milliliter excess. The control is adjusted carefully until the eye is completely open. (The control must not be turned beyond this point.) The standard methanol solution, containing about 4 milligrams of water per milliliter, is added at a rate of 1 to 2 drops per second until the eye starts to close. The methanol then is added dropwise, allowing the necessary time interval between drops for the eye to close when the endpoint is reached. At the endpoint the eye will close completely within 2 to 5 seconds. This lag in reaching maximum polarization is characteristic of all electrometric techniques for the Karl Fischer titration.

TABLE XVII

Analyses for Water in Methanol by "Dead-Stop" Endpoint Method[19]

Water added, mg.	Total water present, mg.	Water found, mg.	Difference, mg.
56.5	68.2	67.3	−0.9
47.7	59.4	59.5	+0.1
55.0	66.7	66.8	+0.1
139.1	150.8	151.3	+0.5
121.4	133.1	132.3	−0.8
123.2	134.9	134.3	−0.6
168.2	179.9	179.5	−0.4
184.6	196.3	196.4	+0.1

The accuracy of the method compares favorably with other procedures. In Table XVII results are tabulated on the analysis of equal volumes of methanol, containing 11.7 milligrams of water, to which weighed quantities of water were added.

Systems similar to those described above have been used widely by those analysts who prefer an electrometric technique.[5,7,10] Several of the scientific apparatus companies are now furnishing titrimeter units adapted to the Fischer reagent titration, to be used in conjunction with a cathode ray tube, galvanometer, or microammeter.

The use of a sensitive microammeter permitted a simplified titrimeter circuit with the power being furnished by a standard 1.5 volt dry cell.[12] The authors[6] used successfully a circuit composed of a 1.5 volt dry cell, 100,000 ohm potentiometer-type resistor with a sensitive microammeter connected as indicated in the original "dead-stop" apparatus (Fig. 20). The potential was adjusted by setting the resistor to give about three-quarter scale deflection on the microammeter (full scale = 20 microamperes) in the presence of excess Fischer reagent. At this setting the current fell to practically zero at the endpoint. As with other indicating instruments the over-all change required several seconds. With care the endpoint could be reproduced to 0.05 ml. of Karl Fischer reagent equivalent to about 0.12 milligram of water. The same differential change by direct titration with Fischer reagent, i.e., zero to about 70 per cent of full-scale, required about 0.4 ml. of reagent. This was in agreement with the observations of other investigators.[3,4]

d. Potentiometric Direct Titration Method

Most investigators of electrometric systems have indicated that the indirect titration method, i.e., addition of excess Karl Fischer reagent followed by back titration with water-in-methanol, tended to give more accurate and precise results. Recently, however, several investigators have presented direct titration schemes which apparently embody an equally high order of precision.[16,20,21,23] Heinemann[20] employed a potentiometric method using the glass–platinum electrode system suggested by Lykken and Tuemmler[35]—the glass electrode served as the reference and the platinum wire as an indicating electrode. In other respects the apparatus was like that of Almy, Griffin, and Wilcox,[3] including the use of a *p*H meter to detect the endpoint. With this system the endpoint was found to be equally sharp whether the solution was titrated directly with

[35] Lykken, L., and Tuemmler, F. D., *Ind. Eng. Chem., Anal. Ed.* **14**, 67–9 (1942).

Karl Fischer reagent to the potentiometric endpoint or an excess of Fischer reagent was added, followed by back titration with standard water-in-methanol. The endpoint was characterized by a sudden change in potential which became stationary for 10 to 15 seconds.

e. Dead-Stop Direct Titration Method

Carter and Williamson,[21] Cornish,[16] and Kieselbach[22] have reported the use of direct "dead-stop" techniques, which are based on a greatly increased potential between the platinum electrodes. In the Carter-Williamson arrangement (Fig. 22a), *R*1 is a high resistance, which may range from 3000 to 10,000 ohms, and the shunt resistance, *R*2, is of the order of 0.025 to 0.1 of the galvanometer resistance. Actual values for *R*1 and *R*2 are best determined by trial titrations and are set so that an observable change is noticed on the galvanometer scale between the current reading during the titration and the surge current reading at the endpoint. For example, these investigators[21] used a 2 volt dry cell and a galvanometer of the mirror type with a sensitivity of 24 millimeters per microampere and with a 100 millimeter scale for the spot movement. The galvanometer resistance was 373 ohms, *R*2 was 10 ohms, and *R*1 was 5000 ohms.

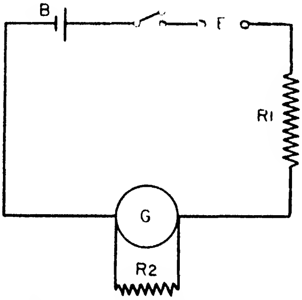


Fig. 22a. Wiring diagram.[21]
B, dry cell
R1, R2, resistors

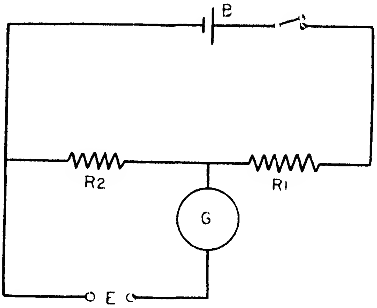


Fig. 22b. Wiring diagram.[16]
G, galvanometer
E, electrodes

The electromotive force applied to the electrodes varied during the titration since the resistance of the system decreased from an initial value of greater than 15,000 ohms to approximately 500 ohms at the endpoint. In methanol solution the potential varied from 1 to 2 volts at the beginning of a titration to about 0.15 volt when the titration was completed. This represented the primary difference between the direct method of Carter and Williamson[21] and the back titration "dead-stop" techniques; the latter, in general, were based on a potential of 10 to 15 millivolts. The galvanometer action in the direct method was, of course, reversed, the current increasing suddenly at the first appearance of unused iodine.

In another modification of the basic "dead-stop" apparatus, a successful direct titration method was reported by Cornish[16] who increased the current from about 5 microamperes to a value of 100 microamperes, while maintaining the applied potential at approximately 10 millivolts. This was accomplished by the use of a 100 ohm (*R*1) and a 5000 ohm resistor (*R*2) with a microammeter (*A*) of 200 ohms resistance reading 0–200 microamperes (see Fig. 22b). The titration cell had a resistance of about 80 ohms; a 2 volt battery (*B*) was used across the platinum electrodes.

In these direct titration schemes only one electrode is polarized (see page 88). As long as water is present the iodine in Karl Fischer reagent reacts, forming hydriodic acid. Since only reductant is present, the anode remains depolarized and the cathode, polarized, so that no current flows. At the endpoint, however, the free iodine depolarizes the cathode, resulting in a surge of current.

In making an analysis Carter and Williamson[21] weighed the sample into a dry titration flask, added a small quantity of dry methanol, and connected the flask to the electrode unit. With the stirrer running, Fischer reagent was added rapidly in 0.2 ml. portions until the endpoint was approached, as evidenced by small but positive oscillations in the galvanometer spot at the beginning of the scale. From this point on the reagent was added dropwise and, when the endpoint was reached, the galvanometer was suddenly deflected to a steady high value, often of the order of one-half full scale. The titers to the endpoint were reproduced to 0.05 ml. Three sets of replicate standardizations, based on weighed quantities of water, are reported in Table XVIII.

Cornish[16] carried out the titration in about the same manner as that just described. The sample, containing about 60 mg. water, was weighed into the titration flask. With the microammeter reading zero, Karl Fischer reagent was added dropwise until, at the endpoint, the pointer moved over to about 90 microamperes.

TABLE XVIII

Standardization of Fischer Reagent by Direct Electrometric Titration[21]

Water added, g.	Fischer reagent, ml.	Factor (g. water/ml. reagent)
0.0265	9.40	0.00282
0.0185	6.55	0.00283
0.0178	6.30	0.00283
0.0271	9.70	0.00280
0.0372	13.40	0.00278
0.0421	15.10	0.00279
0.0472	16.80	0.00281
0.0385	14.09	0.00273
0.0226	8.30	0.00272
0.0268	9.85	0.00272
0.0202	7.40	0.00273

Standardization data on two preparations of Fischer reagent, based on the use of citric acid monohydrate, are given in Table XIX.

TABLE XIX

Standardization of Karl Fischer Reagent[16]

Sample	No.	Citric acid monohydrate, g.	Fischer reagent, ml.	Factor (mg. water/ml.) reagent
A	1	0.896	10.80	7.12
	2	0.9216	11.20	7.08
B	1	0.7458	8.10	7.95
	2	0.8202	8.90	7.92

The sensitivity of the McKinney-Hall circuit (which is shown in Figure 21) was improved by Kieselbach.[22] By means of adding a power transformer and substituting a 5W4 full-wave rectifier with choke for the 6H6 voltage doubler, Kieselbach obtained a sharper image on the cathode ray tube and effectively grounded the circuit. He reported a high order of precision and accuracy in the direct electrometric Karl Fischer titration as demonstrated in the standardization data given in Table XX. In these experiments about 30 ml. of dry methanol were transferred to a 250 ml., standard 24/40 grind, Erlenmeyer flask which was connected to the titration assembly including the modified titrimeter circuit. Karl Fischer reagent was added dropwise until 1 drop caused the eye to remain open for about 30 seconds. A weighed quantity of standard water-in-methanol was added to the "pretitrated" solvent and the titration repeated.

In the direct titration methods, the need for a standard water-in-methanol buret is eliminated and consequently only a Fischer reagent buret is connected to the titration assembly. Based on the published data the endpoint appears to be as sensitive and accurate as that of the indirect procedures.

TABLE XX

Standardization of Karl Fischer Reagent[22]

Water solution,[a] g.	Fischer reagent, ml.	Factor (mg. water/ml. reagent)	Deviation from mean, %
0.8613	30.75	3.265	0.0
0.9231	32.90	3.27	0.2
1.0123	36.22	3.26	0.2
0.8252	29.50	3.26	0.1
0.9138	32.61	3.265	0.1
0.9330	33.33	3.265	0.0
0.9561	34.08	3.27	0.2
0.7196	25.72	3.26	0.1
0.8518	30.39	3.27	0.1
0.8181	29.30	3.255	0.3
	Average	3.265	0.1

[a] 11.66 weight per cent water in methanol.

3. Conclusions on Macrotitration Methods

In the opinion of the authors the visual titration procedure, which employs a minimum of apparatus and is less time-consuming, has been the indicated method for water analysis in general. Only in those cases where deeply colored or insoluble samples completely obscured the endpoint was it necessary to employ electrometric methods. In this laboratory, where all types of organic and inorganic compounds are analyzed, both kinds of equipment are kept available.

D. Microtitrimetric Methods

1. Visual Microtitration Method

In adapting the visual titration for water to a micro scale the authors[86] studied four reagents, the water equivalence of which varied from 3.3 to 0.6 milligrams of water per milliliter of Fischer reagent. (The ratio of iodine to sulfur dioxide to pyridine was maintained at 1 : 3 : 10.) The rates of degradation are shown in Fig. 23. It was evident that as the iodine concentration of the reagent was reduced the rate of decomposition decreased, particularly during the first day following preparation. Below a concentration equivalent to approximately 1 milligram of water per milliliter, however, the endpoint could not be reproduced with sufficient precision to be practical. The 1 milligram reagent was satisfactory, giving an endpoint nearly as sharp as that of the stronger reagent used in macrotitrations. The dilute reagent with its increased sensitivity required more rigid control of the titration technique and more complete protection from atmospheric contamination. The apparatus requirements, indicated in Figure 24, are similar to those of the macro unit

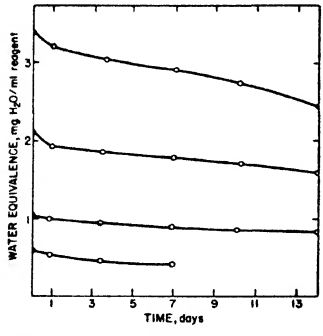


Fig. 23. Rate of loss in strength of Fischer reagent.[86]

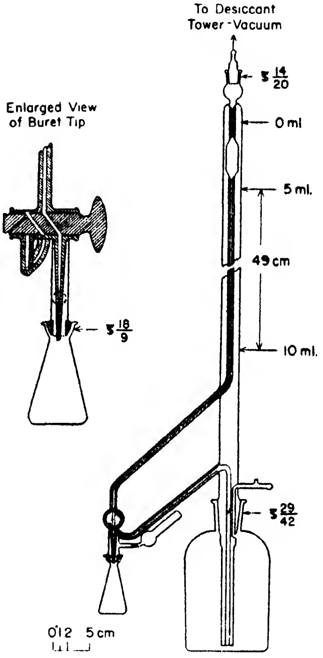


Fig. 24. Buret assembly for visual microtitration.[6]

---

[86] Mitchell, J., Jr., Smith, D. M., and Steelman, F., paper presented before the Division of Analytical and Microchemistry, 106th meeting of the American Chemical Society, Pittsburgh, Pa., September 9, 1943.

(Fig. 6). In the micro equipment an 18/7 inner spherical grind is sealed to the tip of the 10 ml. bottom-filling buret (lower 5 ml. grad. in 0.01 ml.). Desiccant tubes are attached to the side-tube on this grind and also to the 1 liter buret reservoir air inlet. Sample to be titrated is weighed into a dry 25 ml. Erlenmeyer flask with an 18/7 outer spherical grind, using a closed inner member as a stopper. 2 ml. dry CH_3OH are added and the flask is connected to the buret, using a spring clamp to seal the lightly greased spherical grinds. Fischer reagent, equivalent to about 1 milligram of water per milliliter,* is added to the first appearance of unused iodine. (Even though clamped, the titration flask can be agitated continuously during the titration.) The flask is kept in place throughout the titration. The net milliliters of reagent, after correction for the titer of 2 ml. of the dry methanol, is equivalent to the water content of the sample. Analytical data on 2 milliliter portions of methanol to which weighed quantities of water were added are recorded in Table XXI.

TABLE XXI

Micro Determination of Water in Methanol[36]

Water added,[a] mg.	No. of determinations	Water found, mg.
0.85	3	0.85 ± 0.00
1.35	2	1.35 ± 0.00
2.90	2	2.90 ± 0.15
4.00	2	4.00 ± 0.00
4.40	2	4.40 ± 0.00
4.55	6	4.55 ± 0.05
11.35	9	11.35 ± 0.05

[a] Includes water in methanol amounting to 0.3 mg.

The precision and accuracy were good and the sensitivity of about 0.1 milligram of water was considerably better than that of the macro technique which averaged about 0.4 milligram (0.15 ml. of reagent).

2. ELECTROMETRIC MICROTITRATION

Although the visual titration method has been shown to be practical in the quantitative determination of less than milligram quantities of water, the electrometric method is more easily adapted to

---

* One liter quantities of a reagent of this concentration are prepared by dissolving 25.4 grams (0.1 mole) of iodine in 81 ml. (1 mole) of pyridine and then adding 900 ml. of dry methanol. This solution is cooled in a slurry of chopped ice and 13.5 ml. (0.3 mole) of liquid sulfur dioxide are added cautiously.

routine microanalysis. In the latter case manipulative details can be standardized more easily. Maximum precision and accuracy are obtained through the use of Fischer reagent of approximately the same concentration as that employed in the macro methods.

One of the very useful developments in the micro field has been the design of a novel microtitration assembly by Levy, Murtaugh, and Rosenblatt.[14]

Fig. 25. Vessel for electrometric titration.[14]

The titration vessel, Figure 25, consists of a 16 mm. Pyrex test tube cut to a length of about 50 mm. The electrodes, two 26-gage platinum wires, are fused directly through the bottom of the tube. An airtight seal is made by means of a tightly fitting, serum-bottle sleeved rubber stopper. The end of a Schilling type microburet is fitted with a 22-gage hypodermic syringe needle, the end of which is forced through the rubber stopper into the titration vessel. A second 27-gage hypodermic needle acts as a pressure equalizer during the titration. The platinum wire electrodes are connected by means of suitable leads to the titrimeter. This unit is essentially the same as that developed by McKinney and Hall,[19] the only change being the substitution of a 4000 ohm fixed resistor (R_2, Fig. 21). The course of the titration is followed with the "magic eye" cathode ray tube.

Levy and his co-workers also found that a Serfass titrimeter,[34] available commercially, is suitable for the purpose. It is quite probable that electrometric units from other sources can be adapted for use in the micro technique.

In preparing for an analysis the titration flasks are dried and stored over phosphorus pentoxide. Solid samples are weighed in a separate container and transferred rapidly to the dry titration tube which is stoppered immediately. Liquid samples, on the other hand, are introduced into the tared, sealed vessel through a hypodermic needle which is then removed to permit determination of the sample weight.

According to Levy (private communication), for liquid samples of extremely low water content, the flasks are stoppered at about 100°C. to reduce the blank value to a negligible figure. After the flasks have cooled, samples are introduced through hypodermic syringe needles and the titrations made as described above. For small titrations, relief valves are unnecessary. Successful analyses were made on transformer oil containing about 0.01 per cent water.

The microburet and valve are introduced as indicated above and full strength Karl Fischer reagent is added to about 5 per cent excess (determined where possible by the change of color to that of unspent reagent). If necessary, the needles are withdrawn and the vessel shaken until the sample has dissolved or all of the water has been extracted. (In the latter case the additional water extracted may consume the excess iodine; then additional Fischer reagent is added until a small excess remains on standing.) A standard water-in-methanol buret needle, together with the pressure equalizer, is inserted into the rubber stopper and the electrodes are attached to the electrometric apparatus. With the eye of the cathode ray tube in the open position the standard water-in-methanol solution, containing about 2 milligrams of water per milliliter, is added dropwise until the electric eye indicator closes. Because of the flexible rubber stopper the solution may be agitated effectively during the titration by gentle tapping of the vessel. The high precision and accuracy of this technique are demonstrated in the analysis of a sample of ethanol containing 7.65 ± 0.05 per cent water (determined by density using a Bureau of Standards hydrometer), the results of which are shown in Table XXII.

The titration vessel is particularly well suited to the analysis of liquid samples. The required quantity can be transferred to the closed system with a minimum of exposure to the air. Consequently, errors due to outside moisture absorption are minimized.

3. OTHER ELECTROMETRIC MICROTITRATION TECHNIQUES

Microelectrometric methods have been described by Aepli and McCarter[37] and by Acker and Frediani.[38] The former investigators successfully employed the "dead-stop" system of Wernimont and Hopkinson,[4] using Fischer reagent equivalent to 3–5 milligrams of water per milliliter. The latter authors recommended direct electrometric titration with Fischer reagent equivalent to about 2 milli-

---

[37] Aepli, D. T., and McCarter, W. S. W., *Ind. Eng. Chem., Anal. Ed.*, **17**, 316–7 (1945).

[38] Acker, M. M., and Frediani, H. A., *Ibid.*, **17**, 793–4 (1945).

TABLE XXII

Micro Determination of Moisture in Standard Ethanol[14]

Sample weight, mg.	Water found	
	Mg.	Wt. %
312.7	24.0	7.65
238.5	18.25	7.65
296.3	22.65	7.65
305.7	23.4	7.65
306.1	23.4	7.65
298.8	22.85	7.65
326.3	24.75	7.6
267.3	20.5	7.65
299.2	22.8	7.6
279.3	21.35	7.65
	Average	7.65
	Standard deviation	0.025

grams of water per milliliter. The precision of this technique, however, did not measure up to that of the other procedures with which it was possible, in general, to determine the endpoint consistently with a precision of about 0.05 ml. or less on a micro scale and 0.10 ml. or less on a macro scale without undue sacrifice in time or increase in manipulative detail. (Based on the recent improvements in direct electrometric macrotitration methods, it should be possible to devise highly precise direct micro procedures.)

4. Conclusions on Microtitration Methods

The increased precision of electrometric methods makes them preferable for the titration of small quantities of water on a micro scale. In cases where the required apparatus is unavailable, however, it is practical to adapt the visual titration to a micro scale.

CHAPTER V

Determination of Water in Organic Compounds

In discussing the application of the Fischer reagent to the determination of water in organic compounds, reference will be made to other pertinent techniques described briefly in the first chapter. Obviously it is not within the scope of this book to discuss in detail all methods which may be employed for a particular class of compounds. For comparative purposes, however, mention of procedures most commonly employed or techniques having some merit in a particular field appears desirable for a complete understanding of the relative value of the Karl Fischer reagent.

A. General

Karl Fischer[1] described the application of his reagent to the direct titration of water in liquids, solids, and gases, both organic and inorganic. As a result of much laboratory experience with this analytical technique, it is evident that the accurate determination of water is quite practical in nearly all classes of organic compounds. Unlike most of the other techniques, which usually are limited in applicability, the Fischer procedure may be adapted readily to the quantitative analysis of samples, the water content of which may vary from traces to nearly 100 per cent. The successful application of the Fischer reagent, however, requires a thorough knowledge of its limitations particularly with respect to the natures and types of compounds which interfere. Interfering organic substances usually react with one or more of the components of the reagent to form water and thus lead to erroneously high results. In these cases suitable precautions must be taken to eliminate the interfering reactions but, as will be shown later, usually simple and rapid methods may be employed to prepare the samples for the titration.

Occasionally, certain almost anhydrous organic compounds in which the Fischer reagent endproducts are insoluble tend to exhibit a false endpoint after the addition of a small quantity of reagent. By vigorous shaking the iodine color is discharged, after which the

---

[1] Fischer, K., *Angew. Chem.*, 48, 394–6 (1937).

titration may be continued to the complete reaction of all water. This abnormal phenomenon may be eliminated easily by the addition (before titration) of a small quantity of an inert solvent, such as dry methanol, in which both the sample and the reaction products are soluble. Purified dioxane, containing a few thousandths of a per cent of water, provides an outstanding example of this behavior. Addition of the first fraction of a milliliter of Fischer reagent to a 50 or 100 ml. sample of the dioxane gives the deep brown color of unreacted iodine which is discharged only after vigorous shaking. Similar false endpoints have been observed with some halides, hydrocarbons, esters, and alcohols, varying from materials with which water is nearly immiscible to fairly hygroscopic substances. In the latter case the presence of a few per cent of water completely eliminates the false endpoint.

No satisfactory explanation has been offered for the formation of false endpoints. The only commonly associated factor is that of insolubility of one or more of the reaction products, notably pyridine hydroiodide, in the liquid being titrated. The natures of the compounds involved apparently make unlikely the formation of molecular compounds of water. There is no visual evidence of immiscibility with the active Fischer reagent.

In Table XXIII is presented a comprehensive list of organic materials: (*a*) which may be titrated directly, (*b*) which require pretreatment, and (*c*) which at present cannot be analyzed by the Fischer method.

(By direct titration is meant addition of the Fischer reagent to the sample in the presence of an inert solvent such as methanol; in general, macrosamples contained 25 to 250 milligrams of water and, if a solvent was employed, its volume was usually 25 to 50 ml.)

Table XXIII is, in effect, a general summary of the behavior of organic functional groups during direct titrations for water using Karl Fischer reagent. The detailed discussion of organic compounds which follows further defines the broad scope of this versatile reagent for the determination of water.

B. Determination of Water in Organic Acids

Techniques which could be applied generally to the determination of water in organic acids have been limited in number. The acyl chloride and acid anhydride procedures could, of course, be used, but, since in most cases the analysis depended on hydrolysis followed by titration of the free acid formed, it was necessary to apply a correction for acid originally present in the sample. These, therefore, were indirect methods, which became of little value when relatively small quantities of water were involved. Toennies and Elliott[2] eliminated this difficulty by substituting a polarimetric method based on decrease in optical rotation after reaction of excess acetic anhydride with camphoric acid. This technique probably was not of general utility but might be applicable to organic acids, with the possible exception of formic acid.

Organic acids of all types have been analyzed for water by direct titration with Fischer reagent. Karl Fischer in his original publication[1] made no mention of the possible application of his reagent to acidic materials but Zimmermann,[3] in 1939, demonstrated that oxalic acid dihydrate, oleic acid, and sulfosalicylic acid dihydrate could be analyzed for water content by the Fischer method. At about the same time the first of a series of Karl Fischer papers from the authors' laboratory mentioned that carboxylic acids in general could be analyzed without interference.[4]

Formic and acetic acids reacted slowly with the methanol of the reagent to form the corresponding esters and water. Provided that the titration was not prolonged, however, this interference usually was limited. This is illustrated in Table XXIV, showing results obtained on the direct analysis of duplicate samples of aqueous formic acid solutions.[5]

Some acids, because of the ease with which they esterify, tended to give slightly high results when methanol was used as a diluent. This amounted to an appreciable relative error when only traces of water were present. Adipic acid, for example, containing 0.001 per cent water, analyzed 0.002 per cent on a routine basis when a 25 gram sample was titrated in the presence of 50 ml. of methanol. Results of 0.001 ± 0.000 per cent water were obtained when a mixture of equal parts of dry methanol and pyridine was used as

---

[2] Toennies, G., and Elliott, M., *J. Am. Chem. Soc.*, **59**, 902–6 (1937).

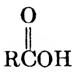
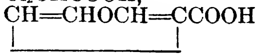
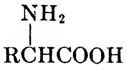
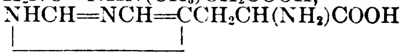
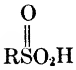
[3] Zimmermann, A., *Fette u. Seifen,* **46**, 446–50 (1939).

[4] Smith, D. M., Bryant, W. M. D., and Mitchell, J., Jr., *J. Am. Chem. Soc.*, **61**, 2407–12 (1939).

[5] Mitchell, J., Jr., and Hawkins, W., *Ibid.*, **66**, 1797 (1944).

TABLE XXIII

Classification of Organic Compounds for Titrimetric Determination of Water

Class	General formula	Examples	Refer to page
(a) Compounds which can be titrated directly			
I. Acids			104
Monobasic carboxylic	 $\mathrm{R}\overset{\mathrm{O}}{\overset{\Vert}{\mathrm{C}}}\mathrm{OH}$	$HCOOH$, CH_3COOH,	105, 113
		$C_{17}H_{35}COOH$,	112
		$Cl_2CHCOOH$,	112
		 $CH{=}CHOCH{=}CCOOH$	112
Polybasic carboxylic	$R(COOH)n$	$(\text{—}COOH)_2$,	105, 158
		$(\text{—}CH_2CH_2COOH)_2$,	105
		$HOOCCH{=}CHCOOH$,	113
		$(HOOCCH_2CH_2)_2S$,	112
		$C_6H_2(COOH)_4$	112, 158
Hydroxy	$HORCOOH$	$HOCH_2COOH$, $(\text{—}CHOHCOOH)_2$,	112
		$HOCCOOH(CH_2COOH)_2$,	112
		$C_{17}H_{32}(OH)COOH$	112
Amino	 $\mathrm{R}\overset{\mathrm{NH_2}}{\overset{\vert}{\mathrm{C}}}\mathrm{HCOOH}$	H_2NCH_2COOH,	112
		$CH_3SCH_2CH(NH)_2COOH$,	112
		$H_2NC{=}NHN(CH_3)CH_2COOH$,	112
		 $NHCH{=}NCH{=}CCH_2CH(NH_2)COOH$	112
Sulfonic	 $\mathrm{R}\overset{\mathrm{O}}{\overset{\Vert}{\mathrm{S}}}\mathrm{O_2H}$	$CH_3{\cdot}C_6H_4{\cdot}SO_3H$,	112, 158
		$H_2N{\cdot}C_6H_4{\cdot}SO_3H$,	112, 158
		$SO_3H{\cdot}C_6H_3(OH)COOH$,	112, 158
		$(CH_3)_2{\cdot}C_6H_3{\cdot}SO_3H$	112, 158

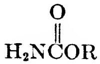
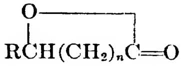
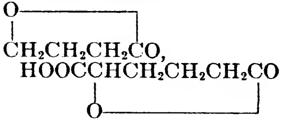
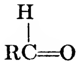
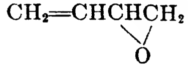
II. Alcohols monohydric	ROH	CH_3OH, $CH_2{=}CHCH_2OH$, $(CH_3)_3COH$, $C_6H_{11}{\cdot}OH$, $CH_2{=}CHC{\equiv}CC(CH_3)_2OH$, $(CH_3)_2C{=}CH(CH_2)_2C(CH_3){=}CHCH_2OH$, $C_{10}H_{19}OH$ (menthol)	114 115, 116 116 117 116 116
Polyhydric	R(OH)*n*	$HOCH_2CH_2OH$, $HOCH_2CH(OH)CH_2OH$, $HOCH_2C{\equiv}CCH_2OH$, $C(CH_2OH)_4$, $HOCH_2(CHOH)_4CH_2OH$	116 116, 117 117 116 116
Phenols	$C_6H_{6-n}(OH)_n$, etc.	$C_6H_5{\cdot}OH$, $C_6H_3(OH)_3$, $(NO_2)C_6H_2{\cdot}OH$, *tert*—$C_4H_9{\cdot}C_6H_3(OH)_2$	158 116, 117 117
III. Esters	 $R\overset{O}{\overset{\Vert}{C}}OR^1$	$HCOOCH_3$, $CH_3COOC_2H_5$, $HCOOCH_2CH{=}CH_2$, $CH_2{=}CHCOOCH_3$	118, 119 119 118
Ortho esters Esters of inorganic acids	$RC(OR')_3$ R_2CO_3, RNO_3, R_2SO_4, etc.	$HC(OC_2H_5)_3$, $CH_3C(OCH_3)_3$, $(C_4H_9)_2CO_3$, $C_4H_9NO_2$, $(CH_3)_2SO_4$, $(C_2H_5)_3PO_4$, $(C_4H_9O)_2SO$, C_2H_5OCOCl	118, 119 118, 120 118, 119 118, 120
Carbamates (urethans)	 $H_2N\overset{O}{\overset{\Vert}{C}}OR$	$H_2NCOOC_2H_5$, $CH_3NHCOOC_2H_5$	119 118
Lactones			119 118
IV. Aldehydes, stable	 $R\overset{\overset{H}{\vert}}{C}{=}O$	HCHO, CCl_3CHO, Sugars (aldoses)	147, 158

Table (continued)

TABLE XXIII (*continued*)

Class	General formula	Examples	Refer to page
V. Ketones, stable	$R_2C{=}O$	$(CH_3)_2CHCOCH(CH_3)_2$,	147
		$C_6H_5{\cdot}COCO{\cdot}C_6H_5$,	147
		$C_6H_5{\cdot}CH(OH)CO{\cdot}C_6H_5$,	147
		Sugars (ketoses)	202
VI. Acetals	$RCH(OR')_2$	$CH_2(OCH_3)_2$,	121
		$CH_2OCH_2OCH_2$,	121
		$CH_3OCH(OCH_3)_2$	121
VII. Ethers	ROR'	$C_2H_5OC_2H_5$, CH_3OCH_3,	121, 122
		$OCH_2CH_2OCH_2CH_2$,	122
		 $CH_2{=}CHCHCH_2$ (O)	122
VIII. Hydrocarbons			121
Saturated	RH	$CH_3(CH_2)_3CH_3$,	121
		$CH_2(CH_2)_4CH_2$	121
Ethylenic	$RCH{=}CHR$	$CH_3CH{=}CHCH_2CH_3$,	121
		$CH_2{=}CHCH{=}CHCH_3$, C_6H_{10},	122
		$CH{=}CHCH(CH_3)CH_2CH_2C{=}C(CH_3)_2$	121
Aromatic	$C_6H_{6-n}R_n$	C_6H_6, $C_6(CH_3)_6$,	121, 123
		$C_6H_5{\cdot}CH{=}CH_2$, $C_{10}H_8$	121, 124
IX. Anhydrides	$(RC{=}O)_2O$	$(CH_3CO)_2O$, $(CH_3CH_2CO)_2O$,	125
		$(C_6H_5CO)_2O$, $C_6H_4(CO)_2O$	125
X. Acyl halides	 $R\overset{O}{\overset{\Vert}{C}}X$	$C_6H_5{\cdot}COCl$,	
		$(NO_2)_2{\cdot}C_6H_3{\cdot}COCl$	125

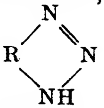
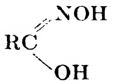
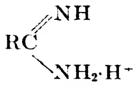
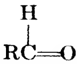
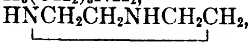
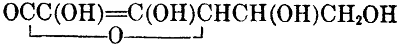
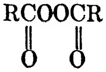
XI. Peroxides			
Hydro	ROOH	$(CH_3)_3COOH$	142
Dialkyl	ROOR	$C_2H_5OOC_2H_5$, $(CH_3)_3COOC(CH_3)_3$	143 143
XII. Neutral, basic and weakly acidic nitrogen compounds			126
Amides	$RCONH_2$	CH_3CONH_2, H_2NCONH_2, $H_2NCONHCONH_2$, $C_6H_5{\cdot}CONH_2$	127 128
Anilides	$C_6H_5{\cdot}NHCOR$	$C_6H_5{\cdot}NHCOCH_3$	128
Amines (K = 2.4 × 10^{-5} or weaker)	RNH_2, R_2NH, R_3N	$(CH_2)_6(NH_2)_2$, $C_6H_5{\cdot}NH_2$, $C_6H_5{\cdot}CH_2NH_2$, C_9H_7N, $CH_3{\cdot}C_6H_4{\cdot}NH_2$, $\underbracket{CH{=}CHCH{=}CHNH}$, $C_6H_{12}N_4$ (hexamethylenetetramine)	128 128 128 128 127
Amino alcohols (alkamines) (K = 2.4 × 10^{-5} or weaker)	$HORNH_2$, $(HOR)_2NH$, $(HOR)_3N$	$H_2N{\cdot}C_6H_4{\cdot}OH$, $HOC_2H_5{\cdot}C_5H_5N$	127 128
Purines (diureides)		$C_5H_4O_3N_4$ (uric acid)	128
Proteins		Trypsin	129
Lactams	$\underbracket{HN(CH_2)_nC{=}O}$	$\underbracket{HN(CH_2)_5CO}$	127
Imines	$RCH{=}NR^1$	$CH_3(CH_2)_2CH{=}N{\cdot}C_6H_5$	127
Azo, azole compounds	$RN{=}NR^1$, 	$\underbracket{C_6H_4NHN{=}N}$, $\underbracket{C_6H_4SCH{=}N}$	126 128
Nitriles	$RC{\equiv}N$	CH_3CN, $CH_2{=}CHCN$, $(-CH_2CH_2CN)_2$, $NCCH_2CH{=}CHCH_2CN$	127 128 127
Cyanohydrins	$R\overset{OH}{\overset{\vert}{C}}HC{\equiv}N$	$HOCH_2CN$, $(CH_3)_2C(OH)CN$, $CH_3CH{=}CHCH(OH)CN$	128, 129 128

Table (*continued*)

TABLE XXIII (*concluded*)

Class	General formula	Examples	Refer to page
Oximes	$RCH{=}NOH$, $R_2C{=}NOH$	$CH_3(CH_2)_2CH{=}NOH$, $(CH_3)_2C{=}NOH$	128 129
Hydroxamic acids	 $RC(=NOH)OH$	$CH_3C(NOH)OH$	128
Amidine salts	 $RC(=NH)NH_2{\cdot}H^+$	$(NH_2)_2C{=}NH{\cdot}HNO_3$	129
Isocyanates	$RN{=}C{=}O$	$C_6H_5{\cdot}NCO$	129
Nitroso compounds	$R_2N{-}N{=}O$	$(C_6H_5)_2NNO$	129
Nitro compounds	RNO_2	CH_3NO_2, $C_6H_4(NO_2)_2$, $(NO_2)_2C_6H_3{\cdot}COOH$	128, 129 128
Cyanic acid derivatives	$HOC{\equiv}N$, $H_2NC{\equiv}N$	H_2NCN, $C_3N_3(NH_2)_3$, $C_3N_3(OH)_3$	129
Alkaloids		Strychnine, brucine	157
XIII. Halides	RX	CH_3Cl, $BrCH_2CH_2Br$, $CH_2{=}C(Cl)CH{=}CH_2$, $CH_2ClCH{=}CHCH_2Cl$	128, 130 132 134
XIV. Sulfur compounds			
Sulfides (thio ethers)	RSR	$(CH_2{=}CHCH_2)_2S$, $(C_4H_9)_2S$	132 134
Disulfides	$RSSR$	$(C_4H_9S)_2$	134
Thiocyanates	$RSCN$	$CH_3(CH_2)_3SCN$, $({-}CH_2SCN)_2$,	134 132
Isothiocyanates	$RNCS$	C_2H_5NCS	132, 134
Thio esters	$RCOSR$	$CH_3COSC_2H_5$	134
(b) Compounds which interfere on direct titration but for which modified techniques are available			
I. Aldehydes, active	 $RC(H){=}O$	CH_3CHO, $C_6H_5{\cdot}CHO$	152
II. Ketones, active	$R_2C{=}O$	CH_3COCH_3, $CH_3COCOOH$	152

III. Amines, stronger than benzylamine ($K = 2.4 \times 10^{-5}$)	RNH_2, R_2NH, R_3N	$CH_3(CH_2)_3NH_2$,	128
		 $\underbrace{HNCH_2CH_2NHCH_2CH_2}$,	159
		$(CH_3CH_2)_3N$	127
IV. Amino alcohols (alkamines)	H_2NROH, $HN(ROH)_2$, $N(ROH)_3$	$H_2NCH_2CH_2OH$,	128
		$HN(CH_2CH_2OH)_2$	128
V. Methylolureas		$HOCH_2NHCONHCH_2OH$	155
VI. Hydrazine salts, substituted hydrazine salts	$RNHNH_2 \cdot H^+$	$(NH_2)_2 \cdot 2HCl$,	131
		$H_2NCONHNH_2 \cdot HCl$,	131
		$C_6H_5 \cdot NHNH_2 \cdot HCl$	131
VII. Mercaptans	RSH	C_4H_9SH, $HSCH_2COOH$	137
VIII. Thiourea and *N*-substituted thioureas	 $RNH\underset{\underset{S}{\Vert}}{C}NHR$	H_2NCSNH_2, $C_6H_5 \cdot NHCSNH_2$,	139
		$C_6H_5 \cdot NHCSNH \cdot C_6H_5$	140
IX. Thioacids	 $R\underset{\underset{O}{\Vert}}{C}SH$, $R\underset{\underset{S}{\Vert}}{C}SH$	CH_3COSH	141
(c) Compounds which interfere and for which no inhibiting techniques are now available			
I. *l*-Ascorbic acid (vitamin C)	 $OCC(OH)=C(OH)CHCH(OH)CH_2OH$		113
II. Quinone	$O=C_6H_4=O$		155
III. Peracids	 $R\underset{\underset{O}{\Vert}}{C}OOH$		146
IV. Peroxides, diacyl	 $R\underset{\underset{O}{\Vert}}{C}OO\underset{\underset{O}{\Vert}}{C}R$	$(CH_3CO)_2O_2$	143

diluent. The inhibiting influence of the amine was sufficient to discourage any esterification of the acid at room temperature.

TABLE XXIV

Determination of Water in Formic Acid[5]

Water found, wt. %	Acid found,[a] wt. %	Total, wt. %
89.85 ± 0.05	10.22 ± 0.01	100.07
70.30 ± 0.10	29.76 ± 0.02	100.06
26.10 ± 0.10	74.00 ± 0.20	100.10
14.80 ± 0.00	85.20 ± 0.00	100.00
1.55 ± 0.05	98.80 ± 0.02	100.35

[a] By titration with standard alkali to phenolphthalein.

A list follows of some of the acids which have been successfully titrated for water. Included are mono- and polybasic carboxylic acids (both saturated and unsaturated), acids containing amino, sulfalkyl, and hydroxyl groups, and sulfonic acids:

Monobasic acids

Formic
Acetic
Mono-, di-, and trichloracetic
Trifluoracetic
Propionic
Acrylic
Methacrylic
Trimethylacetic
Caproic
Stearic
Oleic
Linoleic
Linolenic
Fencholic
Cyclohexanecarboxylic
Cyclohexaneacetic
Camphoric
Abietic
Benzoic
3,5-Dinitrobenzoic
Cinnamic
Anisic
Benzilic
2,4-Dichlorophenoxyacetic (2,4-D)
Furoic

Polybasic acids

Oxalic
Malonic
Maleic
Succinic
Diglycolic
Glutaric
Adipic
Sebacic
Thiodiglycolic
Thiodipropionic
Nitrilotriacetic
Phthalic
Pyromellitic

Sulfonic acids

Benzene
Toluene
Xylene
Naphthalene
Sulfanilic
1,8-Dihydroxynaphthalenedisulfonic

Hydroxy acids

Glycolic
α-Hydroxybutyric
Lactic
Citric
Tartaric
δ-Hydroxycaproic
α-Hydroxyadipic
α, α′-Dihydroxyadipic
Ricinoleic
Salicylic

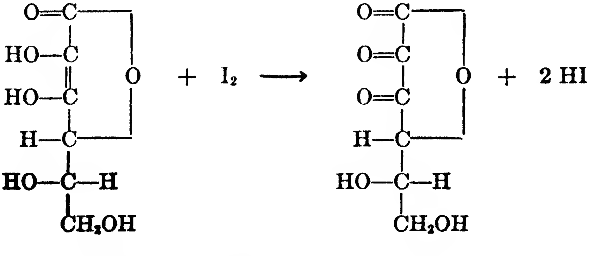
Amino acids

Glycine
Leucine
Sarcosine
Valine
Aspartic
Histidine
Iminodiacetic
Creatine
Asparagine
Methionine
Cystine
Phenylalanine

Levy and his coworkers[6] in a series of 10 determinations on acetic acid samples weighing about 250 milligrams reported an average of 0.66 per cent water with a standard deviation of 0.01. Rennie and Monkman[7] used samples ranging from 1 to 5 milliliters of glacial acetic acid to demonstrate that the average of 0.35 per cent water found (standard deviation = 0.005) represented actual water content. Zimmermann[3] reported a value for water in oleic acid, based on duplicate analyses, of 0.26 ± 0.03 per cent. He concluded that this value was real since he found that on heating the acid in a long tube water was condensed at the cold end.

Analyses have been made on all of the acids listed above in the authors' laboratory on a routine basis. The apparent absolute precision and accuracy varied from ±0.00 to ±0.1 per cent on samples containing from 0.00 to 28 per cent water, depending primarily on the care with which the titrations were made. For example, maleic and linoleic acids, two of the listed unsaturated acids, showed no evidence of interference; duplicate analyses on direct titration for water gave results of 0.24 ± 0.02 and 0.145 ± 0.005 per cent, respectively.[8]

l-Ascorbic acid (vitamin C) apparently reacted quantitatively with Fischer reagent. In the presence of weak oxidizing agents this compound was known to dehydrogenate with ease to dehydroascorbic acid:



(1)

Johnson[9] found that the iodine consumption of this compound was equivalent to that required for equation (1); that is, 1 mole of iodine was used up per mole of ascorbic acid titrated. Apparently the Karl

---

[6] Levy, G. B., Murtaugh, J. J., and Rosenblatt, M., *Ind. Eng. Chem., Anal. Ed.*, **17**, 193–5 (1945).

[7] Rennie, R. P., and Monkman, J. L., *Can. Chem. Process Inds.*, **1945**, 366–70.

[8] Unpublished results from the authors' laboratory.

[9] Johnson, C. M., *Ind. Eng. Chem., Anal. Ed.*, **17**, 312–16 (1945).

Fischer reagent titer, then, was equal to the sum of *l*-ascorbic acid plus water, and water could be determined if there were an independent method for the determination of the ascorbic acid.

C. Determination of Water in Alcohols

Many products involving hydroxylated compounds are obtained in high yield only under relatively anhydrous conditions. In the malonic ester synthesis, for example, the yield of *n*-butyl malonic ester was increased from 66 to 85 per cent simply by the use of sodium ethoxide prepared with 99.5 per cent alcohol instead of 98.4 per cent.[10] Also, in the preparation of phenobarbital, yields of the intermediate, phenyl oxalacetic ester, varied from 70 per cent in alcohol containing 0.3 per cent water to 98 per cent when only about 0.01 per cent water was present.[11] Close control of the water content of glycerol is essential in the manufacture of plastics and explosives where only a few tenths of a per cent of moisture can be tolerated.

Of the many techniques employed for the determination of water in alcohols the Karl Fischer method has been the only one which is generally applicable. Density measurements have long been used in the analysis of methanol, ethanol, and glycerol; with suitable equipment, including devices for rigid control of temperature, they still may be used with full confidence only in those cases where water is the only impurity, assuming that the usual standard reference tables (based on the binary systems) are employed. This has likewise been true of other analyses based on a physical measurement, such as refractive index,[12] conductivity,[13,14] boiling point,[15] and critical solution temperature.[16-19] (A number of ternary systems are reported in the literature whereby the water content can be estimated with fair accuracy by the use of two physical measurements, usually density and refractive index.)

Azeotropic distillation methods have found wide use in the determination of water in certain alcohols, particularly glycerol. In

---

[10] Inglis, J. K. H., *Org. Syntheses,* Coll. Vol. I, 249–51 (1932).

[11] Gyngell, E. S., Phillips, M. A., and Smith, E. L., *Industrial Chem.,* 1945, 526–32.

[12] Hoyt, L. F., *Ind. Eng. Chem.,* 26, 329–32 (1934).

[13] Schmidt, M. R., and Jones, H. C., *Am. Chem. J.,* 42, 37–95 (1909).

[14] Kamenyama, N., and Semba, T., *J. Soc. Chem. Ind. Japan,* 30, 10–14 (1927).

[15] Grün, A., and Wirth, T., *Z. angew. Chem.,* 32, 59–62 (1919).

[16] Crismer, L., *Bull. soc. chim. Belg.,* 32, 641 (1904).

[17] Andrews, L. W., *J. Am. Chem. Soc.,* 30, 353–60 (1908).

[18] Osborne, N. S., McKelvey, E. C., and Bearce, H. W., *Bull. Bureau of Standards,* 9, 344 (1913).

[19] Robertson, G. R., *Ind. Eng. Chem., Anal. Ed.,* 15, 451–2 (1943).

general these were based on the Dean-Stark[20] technique employing high-boiling hydrocarbons[21,22] or halides[23] as carriers.

The vacuum desiccation method of Rojahn[24] for the analysis of glycerol solutions, employing phosphorus pentoxide, was shown to be only approximate by Spaeth and Hutchison,[25] who recommended instead a vacuum distillation technique.

Chemical methods, in general, were subject to interference from the alcohol particularly when only small quantities of water in high concentrations of the alcohol were involved. The relatively mild conditions employed by Smith and Bryant[26] in their acetyl chloride hydrolysis procedure were applicable in most cases; high concentrations of the lower alcohols led to erroneous results. The sodium-ester method[11,27,28] was accurate for small quantities of water in methanol and ethanol but was time consuming and indirect. The Karl Fischer method, in addition to being generally applicable, has been the only technique which combines speed with high precision and accuracy in the determination of water in alcohols. Typical compounds which have been analyzed without difficulty are shown in the table on page 116.

Karl Fischer[1] reported quantitative recovery of a known weight of water added to methanol. The calcium hydride method which he used for obtaining comparative data in general could not be used in this case because of the rapid interfering reaction of the alcohol. Wernimont and Hopkinson[29] analyzed several samples of ethanol containing known quantities of water ranging from 0.28 to 7.52 per cent. Based on duplicate analyses, values within 0.01 per cent were obtained in most cases. Scott[30] reported a value of 0.22 per cent water in absolute ethanol by titration, compared to 0.25 per cent based on density. Commercial 95 per cent alcohol dried with calcium

---

[20] Dean, E. W., and Stark, D. D., *Ind. Eng. Chem.*, 12, 486–90 (1920).
[21] Hoyt, L. F., and Clark, P. C., *Oil and Fat Inds.*, 8, 59–61 (1931).
[22] Spiehl-Striemann, *Z. angew. Chem.*, 40, 464–6 (1927).
[23] Berth, T., *Chem.-Ztg.*, 51, 975–6 (1927).
[24] Rojahn, C. A., *Z. anal. Chem.*, 58, 433–42 (1919).
[25] Spaeth, C. P., and Hutchison, G. F., *Ind. Eng. Chem., Anal. Ed.*, 8, 29–32 (1936).
[26] Smith, D. M., and Bryant, W. M. D., *J. Am. Chem. Soc.*, 57, 841–5 (1935).
[27] Gyngell, E. S., *J. Chem. Soc.*, 1926, 2484.
[28] Smith, E. L., *Ibid.*, 1927, 1284–8.
[29] Wernimont, G., and Hopkinson, F. J., *Ind. Eng. Chem., Anal. Ed.*, 15, 272–4 (1943).
[30] Scott, T. A., Jr., *J. Phys. Chem.*, 50, 406–12 (1946).

Monohydric alcohols	Polyhydric alcohols	Phenols
Methanol	Ethylene glycol	Phenol
Ethanol	1,2-Propanediol	Catechol
Isopropanol	1,3-Propanediol	Resorcinol
Allyl alcohol	Glycerol	Hydroquinone
n-, *iso*-, *sec*-and *tert*-Butanol	1,4-Butanediol	Pyrogallol
Isoamyl alcohol	2,3-Butanediol	Phloroglucinol
2-Methyl-4-butanol	2-Methyl-1,3-propanediol	Guaiacol
2,4-Dimethyl-3-pentanol	Diethylene glycol	Picric acid
Vinylethynyldimethylmethanol	1,4-Butynediol	*o*-Cresol
Decanol	Pentaerythritol	*p*-Cresol
Dodecanol	Neopentyl alcohol	Eugenol
Octadecanol	Mannitol	*p*-*tert*-Butylcatechol
Geraniol	Sorbitol	α-Naphthol
Citronellol		β-Naphthol
Cyclohexanol		
Menthol		
Borneol		
Terpineol		
Benzyl alcohol		
Cholesterol		

oxide and distilled through a Podbielniak column analyzed 1.40 and 1.42 per cent water by Fischer reagent titration and density, respectively.

Gyngell and his coworkers[11] in a review of the use of anhydrous alcohol in organic syntheses concluded that the Fischer titration and sodium–ester methods are best suited for water determinations. Their recommended sample size, calling for about 0.5 gram of water, is probably in error since this would require a titration of over 150 ml. of Fischer reagent. A sample containing about 0.05 gram of water would seem more likely.

Berkengeim[30a] applied the Fischer reagent titration to solubility studies of water in several organic compounds. His data for *n*-butanol (B.P. 116.5° to 117.0°C.) were:

Temperature, °C.	Water found, wt. %	Temperature, °C.	Water found, wt. %
−18	2.80	+20	23.1
− 7	12.5	+40	26.4

Zimmermann's[3] analyses, which included results for ethylene and propylene glycol and glycerol, showed a high order of precision; for example, he reported 14.76 ± 0.03 per cent and 0.275 ± 0.015

[30a] Berkengeim, T. I., *Zavodskaya Lab.*, 10, 592–4 (1941).

per cent for ethylene glycol, 0.86 ± 0.04 per cent for propylene glycol, 12.43 ± 0.03 per cent and 0.57 ± 0.00 per cent for glycerol. These data did not provide a satisfactory measure of the accuracy of the method, however, since Zimmermann's calculated water contents involved a summation of analytical errors. McKinney and Hall[31] found 2.6 per cent water in glycerol both by the Fischer titration and by specific gravity. Monkman[32] in a more thorough study found 5.34 ± 0.04 per cent water for 6 titrations of glycerol with Fischer reagent, which compared favorably with 5.30 ± 0.08 per cent water from 4 determinations by specific gravity and 5.18 ± 0.27 per cent water from 5 analyses by the time-consuming distillation procedure. Other investigators have reported on the successful use of the Fischer reagent for the determination of water in monohydric and polyhydric alcohols.[4,33-37*] (Several authors[30,36,37] have demonstrated the value of the Fischer reagent in the accurate evaluation of vapor–liquid equilibria and phase relationships involving alcohols.)

No evidence of interference was observed in the titration of the extremely active alcohols, vinylethynyldimethylmethanol, $CH_2{=}CHC{\equiv}CC(CH_3)_2OH$, and butynediol, $HOCH_2C{\equiv}CCH_2OH$. The former analyzed 0.16 ± 0.00 per cent water and the latter, 0.70 ± 0.01 per cent. After the addition of 0.89 and 1.39 per cent water (including that originally found), these compounds analyzed 0.90 ± 0.01 and 1.41 ± 0.02 per cent water, respectively.[8] Moisture in picric acid could be determined by direct titration. Analyses in triplicate of a moist sample of the c.p. acid gave values of 0.88 ± 0.02 per cent. After an additional calculated 5.00 per cent water was added, results of 5.90 ± 0.05 per cent water were obtained.[8] Equally satisfactory results were observed with *p*-tert-butylcatechol. Initially 0.24 ± 0.00 per cent water was found; when the

---

[*] In this chapter no specific data are reported for the sugars and other carbohydrates which are considered in detail in Chapter VI.

[31] McKinney, C. D., Jr., and Hall, R. T., *Ind. Eng. Chem., Anal. Ed.,* **15,** 460–2 (1943).

[32] Monkman, J. L., *Can. Chem. Process Inds.,* **1945,** 654.

[33] Kaufmann, H. P., and Funke, S., *Fette u. Seifen,* **44,** 345–6 (1937).

[34] Almy, E. G., Griffin, W. C., and Wilcox, C. S., *Ind. Eng. Chem., Anal. Ed.,* **12,** 392–6 (1940).

[35] Pohle, W. D., and Mehlenbacher, V. C., *Oil & Soap,* **23,** 48–50 (1946).

[36] Hafslund, E. R., and Lovell, C. L., *Ind. Eng. Chem.,* **38,** 556–9 (1946).

[37] Fritzsche, R. H., and Stockton, D. L., *Ibid.,* **38,** 737–40 (1946).

water content was increased to a calculated 1.08 per cent, the analyses indicated 1.06 ± 0.01 per cent.

D. Determination of Water in Esters, Lactones, Carbamates, Ortho Esters, and Esters of Inorganic Acids*

The Karl Fischer reagent has provided the only technique which is generally applicable to the determination of water in esters and related materials. Most of the other methods are impractical for the lower esters, usually because of the high vapor pressure or ease of hydrolysis of these compounds. Little experimental data have been published on the analysis of esters for water although several investigators have indicated that the Fischer technique was applicable.[4,29] In reporting that ethyl acetate could be analyzed for water content by the Fischer method, Kaufmann and Funke[33] stated that no other suitable method was known for this particular compound. This statement, if applied to the lower formic esters, would be reasonable; ethyl acetate could be analyzed for water content by other chemical methods, such as the acetyl chloride procedure,[26] which involve hydrolysis of the reagent under relatively mild conditions. Representative esters, lactones, carbamates, ortho esters, and esters of inorganic acids on which quantitative analyses for water have been obtained in the authors' laboratory include the following:

Esters

Methyl formate
Methyl acetate
Ethyl acetate
Allyl formate
Methyl acrylate
Methyl methacrylate
Ethyl malonate (malonic ester)
Methyl valerate
Methyl adipate
Methyl sebacate
Methyl glycolate
2,3-Butylene diacetate
Ethyl citrate
Glycol ricinoleate
Isobutyl isobutyrate
Cyclohexyl acetate
Methyl benzoate
Methyl salicylate
Phenyl acetate

Lactones

γ-Butyrolactone
δ-Adipolactone
Glucono-δ-lactone

Carbamates

Methyl carbamate
Ethyl carbamate (urethan)
Ethyl *N*-methyl carbamate

Ortho esters

Ethyl orthoformate
Methyl orthoacetate
Butyl orthoformate
Methyl ortho-*n*-valerate
Methyl orthopropionate
Methyl ortho-*n*-butyrate

Esters of inorganic acids

Methyl hydrogen sulfate
Dimethyl sulfate
Diethyl sulfate
Ethyl carbonate
Ethyl chlorocarbonate
n-Butyl carbonate
n-Butyl nitrite
n-Butyl sulfite
Triethyl phosphate

---

* See section H for thio esters.

Methyl formate, in common with other formates, could be titrated directly, as shown by analyses on various samples containing water in concentrations varying from a few hundredths of a per cent to several per cent. A ternary mixture of methyl acetate, methanol, and water analyzed 9.80 ± 0.02 per cent water, as compared to 9.7 per cent calculated by difference after analyses for ester and alcohol.[8] Absolute ethyl acetate has been found to contain 0.098 ± 0.002 per cent water initially and 1.759 per cent *versus* 1.764 per cent calculated after the addition of a known quantity of water.[8] Monomeric methyl acrylate, a typical unsaturated ester, analyzed 0.15 ± 0.01 per cent water by direct analysis in triplicate and 3.62 ± 0.00 per cent *versus* 3.63 per cent water calculated after a known quantity of water had been added.[8] Commercial C.P. malonic ester has been found to contain quantities of water varying from 0.05 to 1.00 per cent.[8] (The moisture concentration is a determining factor on yield in malonic ester condensations.) Levy, Murtaugh, and Rosenblatt[6] in a series of 10 analyses of 2,3-butylene diacetate obtained an average value of 2.17 per cent water with a standard deviation of 0.014 by their micro technique.

Lactones, as exemplified by γ-butyrolactone, could be titrated directly. Duplicate samples of this compound initially analyzed 0.13 ± 0.01 per cent and then 2.38 ± 0.01 per cent, after a calculated 2.36 per cent water was added, including that originally found.[8]

The carbamates and ortho esters have consistently given reliable results, exemplified by quadruplicate analyses on urethan of 0.05 ± 0.01 per cent and on ethyl orthoformate of 0.00 ± 0.00 per cent. This has also been true with all esters of inorganic acids which have been tested. Any interference in compounds of this type should have involved the formation of water which would be characterized by rapidly fading endpoints and abnormally high results. Sharp, permanent endpoints have been observed consistently. Nearly anhydrous dimethyl sulfate when titrated directly tended to exhibit a false endpoint. This was eliminated by solution of the sample in 10 volumes of dry methanol just before titration. Duplicate samples analyzed 0.04 ± 0.00 per cent water initially and 0.74 ± 0.00 per cent after sufficient water was added to give a total calculated 0.74 per cent. Diethyl sulfate likewise exhibited a slight false endpoint on direct titration. This was eliminated by solution in a 5 : 1 volume excess of dry methanol before titration. In this environment the analytical results were 0.08 ± 0.00 per cent water initially present, followed by 0.90 ± 0.02 per cent found *versus* 0.89 per cent calculated. Samples of ethyl chlorocarbonate and butyl nitrite were found to contain 0.05 ± 0.005 and 0.075 ± 0.005 per cent water, respectively.

E. Determination of Water in Acetals, Ethers, and Hydrocarbons

The stability of acetals, ethers, and hydrocarbons has rendered them suitable for analysis by a number of general methods. Although acetals may absorb water under strongly acidic conditions to form free carbonyl compounds, the rapidity with which the reagents commonly employed react with water (thereby rendering the solution anhydrous) has minimized any errors from this source. In addition to the use of Fischer reagent the methods most commonly employed for the determination of water in compounds of these types have included distillation, reaction with calcium hydride, calcium carbide, or magnesium nitride, and hydrolysis of acid anhydrides or acyl chlorides. (These techniques are discussed in Chap. I.) The calcium hydride method was used by Fischer[1] as a standard in checking the precision and accuracy of his reagent in the analysis of organic compounds, principally hydrocarbons.

Some indication already has been given that the Fischer reagent may be used for the direct analysis of water in unsaturated acids, alcohols, and esters. Fischer[1] made this rather surprising observation in analyzing cracked benzene having an iodine number of 132, but his experimental results—although indicating no appreciable addition of iodine to the double bond—appeared to be slightly high (0.019 per cent as compared to a minimum of 0.007 per cent by the calcium hydride method.)

Fischer[1] reported that higher values were obtained by the calcium hydride method when the samples were allowed to react for a long time with vigorous stirring. However, he gave no further analytical data.

More conclusive data were furnished by McKinney and Hall[31] who added a 10 ml. excess of Fischer reagent to pine oil samples, after which the mixtures were allowed to stand for 14 minutes before back titrating; their value of 0.67 per cent water was only 0.02 per cent higher than their result obtained by the normal titration. Almy and his coworkers[34] inferred that very active unsaturates interfered with the Fischer method but gave no examples. Within the experience of the authors, there has been no evidence of iodine substitution (to within ± 0.05 per cent) on any of the many unsaturated compounds studied. The following is a list of some of the ethers, acetals, and hydrocarbons which have been analyzed for water without difficulty in the authors' laboratory:

Acetals	Ethers	Hydrocarbons	
Methylal	Dimethyl ether	Propane	Methyl cyclopentane
Acetal	Diethyl ether	Butane	Cyclohexane
Ethylal	Ethylene oxide	Pentane	Cyclohexene
Propylal	Tetramethylene oxide	β-*n*-Amylene	Methylcyclohexane
Dichloroethylmethylal	Butadiene monoxide	1,3-Pentadiene	Benzene
1,3-Dioxolane	Isopropyl ether	2,4-Dimethylpentane	Toluene
Methoxymethylal	Methyl Cellosolve	2,2-Dimethylpentane	Xylene
Dibutoxymethane	Cellosolve	Octane	Styrene
	Methyl carbitol	Isooctane	Ethylbenzene
	Carbitol	Caprylene	Mesitylene
	Diethylene glycol	Isooctene	Naphthalene
	Diphenyl ether	2,2,4-Trimethylpentane	Diphenyl
	1,4-Dioxane	3-Methylhexane	Decalin
	Tetramethyldioxane	Decane	Hexamethylbenzene
		Octadecane	Anthracene
		Dipentene	Phenanthrene
		Cyclopentane	

Analyses of the methanol–methylal azeotrope (92 per cent methylal) gave values of 0.18 ± 0.01 per cent water. A sample of 1,3-dioxolane, analyzing 0.002 ± 0.000 per cent water by direct titration, gave values of 2.32 ± 0.02 per cent as compared to a calculated 2.32 per cent based on the addition of a known quantity of water.[8]

Titrations with the Fischer reagent have been made over a considerable range in temperature. With suitable precautions against the introduction of outside moisture, the titration has been carried out at temperatures considerably below that of the room. Dimethyl ether (B.P. —23.6°C.) was titrated with difficulty, apparently because of a rather stable molecular combination with water. Analyses carried out in methanol at —40° to —60°C., using a closed system of the type shown in Figures 6 and 27, were hampered by the very slow reaction of the water; an initial apparent endpoint was reached which was insufficient to account for the water present in the methanol alone. Only after vigorous shaking was the iodine color discharged, after which a final permanent endpoint was reached.

Water has been titrated successfully at reduced temperatures in other solutions, notably in methyl chloride and dimethylol urea as described later in this chapter. The rate of reaction in these cases was more rapid than in the presence of dimethyl ether.

On the other hand, dimethyl ether, when added to "pretitrated" methanol at —40°C., on titration immediately discharged the iodine color, permitting slow titration to a permanent endpoint. Under these conditions duplicate values of 0.013 ± 0.002 per cent water were found.[8] This ether was best analyzed by means of the apparatus employed for the determination of moisture in gases (Fig. 30, Chapter VI). With this apparatus values of 0.012 ± 0.002 per cent water were obtained.[8] Ethyl ether, as well as all other ethers investigated, could be titrated without any evidence of a false endpoint. A sample of anhydrous ether, analyzing 0.007 ± 0.000 per cent water, gave a value of 0.192 per cent after a further 0.189 per cent had been added.[8] In both cases the ether, dissolved in dry methanol at room temperature, was titrated rapidly to a permanent endpoint. Berkengeim[30a] determined the solubility of water in isopropyl ether ($d_4^{20} = 0.7398$) over a wide range of temperature:

Temperature, °C.	Water found, wt. %	Temperature, °C.	Water found, wt. %
−20	0.64	+10	0.80
−13	0.70	+20	0.87
0	0.75	+40	0.91

Comparative experiments with dioxane were described on page 79 in the previous chapter. Multiple analyses of tetramethyldioxane have been reported by Levy and his coworkers[6] who obtained an average value of 0.79 per cent water with a standard deviation of 0.01 for 10 determinations. Butadiene monoxide, analyzed in triplicate, gave 0.23 ± 0.01 per cent water.[8] The ethers, in general, like the hydrocarbons, were poor solvents for the Fischer reagent endproducts; it usually was essential that methanol or a similar solvent be used as diluent.

Gester[37a] extracted water from hydrocarbons with fractionated

[37a] Gester, G. C., Jr., *Chem. Eng. Progress, Trans.*, 1, 117–22 (1947).

ethylene glycol. Since the glycol usually was immiscible with the hydrocarbon, he was able to determine extremely small quantities of water by extracting large volumes of sample. For example, 250 ml. of glycol was used to extract the water from 1 gallon of hexane. Gester[37a] used the Karl Fischer method with glycol extraction to determine the solubility of water in hexane at temperatures from 50° to 190°F. and also to estimate the equilibrium moisture in hexane at 25°C. after various treatments with solid desiccants:

Desiccant	Equilibrium water in hexane, ppm.	Desiccant	Equilibrium water in hexane, ppm.
$CaCl_2$ (fused)	1.97	Al_2O_3	0.02
$CaCl_2$ (granular)	0.8	KOH (fused)	0.01
CaO	1.1	P_2O_5	0.0001
NaOH (fused)	0.87		

Berkengeim[30a] included a few hydrocarbons in his water solubility studies:

Hydrocarbon	Temperature, °C.	Water found, wt. %
Isooctane		
(d_4^{20} = 0.6947)	−1.5	0.001
	+10	0.004
	+30	0.0055
	+40	0.008
Cyclohexane		
(B.P. 81°C.)	+20	0.009
	+50	0.015
Benzene		
(B.P. 80.4°C.)	+10	0.040
	+20	0.053
	+30	0.066
	+40	0.084
	+50	0.114

Karl Fischer[1] conclusively demonstrated the applicability of his reagent to the analysis of benzene: a sample of benzene containing 0.034 per cent water analyzed 0.031 per cent; another benzene sample analyzed 0.043 per cent water by titration, as compared to 0.046 per cent by the calcium hydride method. A third sample which analyzed 0.016 per cent by titration gave a minimum of 0.009 per cent with calcium hydride. In the latter case Fischer reported that higher values were obtained when the calcium hydride was allowed to react for a long time with vigorous stirring but he gave no further analytical data. A typical diene, 1,3-pentadiene, was titrated directly for moisture; duplicate 25 ml. samples dissolved in 50 ml. of dry methanol gave values of 0.090 ± 0.005 per cent. To a third sample approximately a 15 ml. excess of Fischer reagent was added and, after 30 minutes at room temperature, the solution was back titrated with standard water-in-methanol; the net iodine consumption equivalent to 0.092 per cent water compared favorably with the value obtained on direct titration with Fischer reagent, demonstrating that there was no iodine addition to the diene.[8]

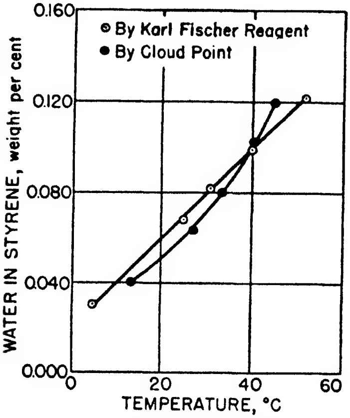


Fig. 26. Solubility of water in styrene.[38]

In making his solubility studies of the styrene–water system, Lane[38] found that the Karl Fischer reagent titrations were highly precise; the difference between duplicate determinations never exceeded 3 per cent of the average value. Solubility data are given in Figure 26 for water in styrene at temperatures ranging from 6° to 51°C., comparing the Fischer reagent titration with a less precise cloud point method.

Compounds containing acetylenic bondings have given no evidence of interference as indicated in the titrations of the alcohols, butynediol, and vinylethynyldimethanol (see page 117).

F. Determination of Water in Anhydrides and Acyl Halides

In the absence of a catalyst or acid acceptor the rate of hydrolysis of acid anhydrides and acyl halides was usually relatively slow. This has been demonstrated in some of the chemical procedures for the determination of water or alcoholic hydroxyl in

---

[38] Lane, W. H., *Ind. Eng. Chem., Anal. Ed.,* 18, 295–6 (1946).

which the excess reagent was hydrolyzed only after extended heating with excess water.[39–41]

Some experiments on the dehydration of glacial acetic acid by means of the anhydride are of interest. The acid containing about 0.5 per cent of water was mixed with slightly more than the calculated amount of acetic anhydride required to produce anhydrous acid. The mixture was allowed to stand at room temperature and was analyzed at intervals by the Karl Fischer procedure. The following results were obtained:[42]

Time, standing at room temperature	Water, wt. %	Time, standing at room temperature	Water, wt. %
0	0.54	4.5 hours	0.36
4 minutes	0.52	21 hours	0.08
1.5 hours	0.47	48 hours	0.015

The reaction was slow but proceeded to completion without a catalyst.

Several anhydrides and chlorides were titrated for water with Karl Fischer reagent. Since in all cases the materials were found to be anhydrous, further analyses were made after a known quantity of water was added. To approximately 5 gram samples of each of the compounds were added 25 ml. portions of acetic acid containing 95.0 milligrams of water at room temperature (28°C.). The solutions were titrated immediately with Karl Fischer reagent. Results are given in Table XXV. The two lower anhydrides indicated some hydrolysis. Similar titrations carried out at 15°C. gave recoveries of 94.4 and 94.7 milligrams of water on acetic and propionic

TABLE XXV

Recovery of Water Added to Anhydrous Acid Anhydrides and Acyl Chlorides[8]

Substance	Water found, mg.[a]	Substance	Water found, mg.[a]
Acetic anhydride	92.8	Benzoic anhydride	95.0
Propionic anhydride	93.7	Benzoyl chloride	94.7
Phthalic anhydride	94.4	3,5-Dinitrobenzoyl chloride	95.1

[a] Water added = 95.0 mg.

---

[39] Ross, J., *J. Soc. Chem. Ind.*, 51, 121–2T (1932).
[40] Nieuwenburg, C. J., *Chem. Weekblad,* 34, 217 (1937).
[41] Lewkowitsch, J., *J. Soc. Chem. Ind.*, 16, 503–6 (1897); 9, 842–8 (1890).
[42] Smith, D. M., Bryant, W. M. D., and Mitchell, J., Jr., *J. Am. Chem. Soc.*, 62, 608–9 (1940).

anhydrides, respectively.[8] At 0°C. the recovery of water from these anhydrides was quantitative.

G. Determination of Water in Basic, Neutral, or Slightly Acidic Organic Nitrogen Compounds

These important classes of compounds include amides, anilides, amines, alkaloids, proteins, amino acids (discussed with acids), carbamates or urethans (discussed with esters), lactams, cyanohydrins, nitriles, nitro, nitroso, azo, and azole compounds, oximes, hydrazines, hydroxamic acids, alkamines or amino alcohols, isocyanates, purines or diureides, amidines, and cyanic acid derivatives. Techniques which have been applicable to the determination of water in organic nitrogen compounds were generally limited in scope. Chemical methods based on hydrolysis of acyl chlorides or acid anhydrides were sometimes applicable, but not to those substances—notably amines and amides—which underwent interfering acylation reactions or which interfered with the normal acidimetric endpoint. (With all but the lower amines the relatively mild acetyl chloride procedure of Smith and Bryant[26] should be applicable.) In the specific case of aniline, methods have been reported which were based on cloud point.[43-45]

All of the types of organic nitrogen compounds that are listed above have been titrated for water by means of Fischer reagent. Amines stronger than benzylamine ($K = 2.4 \times 10^{-5}$) were preferably treated before titration with an excess of glacial acetic acid or spent Fischer reagent, which in both cases combined rapidly with the amines. In the absence of either of these acidic reagents, slightly high results were common with strong amines containing small quantities of water. Butylamine, for example, containing 1.8 per cent water, tended to give results from 0.1 to 0.3 per cent high. (Where methanol was used as a diluent in these cases, the amine reacted vigorously with the acidic components of the reagent resulting in a dense vapor above the liquid in the flask, which tended to obscure the endpoint. Weakly basic compounds such as *o*-phenylenediamine, *p*-aminoazobenzene, and diazoaminobenzene, tend to give rather dark solutions when titrated in methanol diluent; for

---

[43] Jansen, J. D., and Schut, W., *Chem. Weekblad,* 20, 657–8 (1923).
[44] Griswold, J., *Ind. Eng. Chem., Anal. Ed.,* 12, 89–90 (1940).
[45] Seaman, W., Norton, A. R., and Hugonet, J. J., *Ibid.,* 15, 322–4 (1943).

visual titration, the lighter colored acetic solution of these amines is preferable. Methanol, on the other hand, is suitable as a solvent for all other classes of nitrogen compounds studied. With the exception of the two proteins[6,31] all of the nitrogen compounds listed (p. 128) were analyzed in the authors' laboratory. Acetic acid has been used as solvent for all amines and is recommended for them in general.[4]

The high precision obtained with acetamide (0.13 ± 0.01 per cent in 10 determinations) was representative of the precision to be expected with compounds of this type.[8] In analyzing urea, one sample in methanol solution was analyzed directly and a second was analyzed after addition of a known quantity of water. Portions of widely different weights of the first sample analyzed 0.04 ± 0.01 per cent water and of the second, 6.85 ± 0.02.[8] The latter sample contained 6.87 per cent, including the water found in the original sample. Ethyleneurea gave values of 0.075 ± 0.004 per cent water on titration of quadruplicate samples with Fischer reagent. An aqueous solution of triethylamine analyzed 70.5 ± 0.2 per cent water and 29.4 ± 0.1 per cent amine by titration with standard acid to the bromphenol blue endpoint, while ethylenediamine was found to contain 39.6 ± 0.1 per cent water and 60.4 ± 0.1 per cent amine.[8] Hexamethylenetetramine gave a weight loss, after 21 hours' desiccation over concentrated sulfuric acid, of 0.05 per cent and 0.22 ± 0.01 per cent water in 6 determinations by Karl Fischer reagent. The desiccated material indicated an additional 0.17 per cent water by titration. A sample of anhydrous hexamethylenetetramine analyzed 0.00 per cent water by Fischer reagent titration.[8] Duplicate samples of monoethanolamine analyzed 1.3 ± 0.0 per cent water initially and 4.7 ± 0.1 after an additional 3.3 per cent had been added, while similar analyses on *m*-aminophenol were 0.10 ± 0.01 per cent and 1.3 ± 0.1 per cent water compared to 1.4 per cent calculated.

A sample of ϵ-caprolactam gave values of 0.37 ± 0.00 per cent water and salicylalethylenediimine, 0.46 ± 0.01 per cent. Acetonitrile analyzed 1.66 ± 0.03 per cent water in quadruplicate determinations. After the addition of a known quantity of water, 4.78 ± 0.02 per cent was found *versus* 4.76 calculated. 1,4-Dicyano-2-butene and methyleneaminoacetonitrile, both unsaturated compounds, gave results of 0.08 ± 0.00 and 0.00 ± 0.00 per cent water, respectively, on titration of duplicate samples with Fischer reagent.

Amides, Anilides

Formamide
Acetamide
Malonamide
Dimethylformamide
Glutaramide
Adipamide
Urea
Ethyleneurea
Biuret
Acetylurea
Alloxan
Benzamide
Salicylamide
Furoamide
Acetanilide
Propionanilide

Purines (diureides), proteins

Uric acid
Barbituric acid
Dimethylhydantoin
Trypsin
Protein (unidentified)

Lactam, imines

ε-Caprolactam
Benzal-*n*-butylimine
Salicylalethylenediimine

Cyanohydrins

Formaldehyde cyanohydrin
Acetone cyanohydrin
Acrolein cyanohydrin

Azo, azole compounds

p-Aminoazobenzene[a]
Diazoaminobenzene[a]
Benzotriazole

Alkaloids

Brucine
Strychnine
Aloin
Berberine sulfate
Betaine hydrochloride

Amines

Ethylamine[a]
Ethylenediamine[a]
Trimethylamine[a]
Propylamine[a]
Propylenediamine[a]
Tetramethylammonium bromide
Diethylamine[a]
Butylamine[a]
Diethylenetriamine
Hexamethylenediamine
Triethylamine[a]
Diisobutylamine[a]
Di-*sec*-butylamine[a]
Cyclohexylamine
o-Aminodicyclohexyl
Aniline
Benzylamine
Methylaniline
Dimethylaniline
p-Bromoaniline
Toluidine
Carbazole
o-Phenylenediamine[a]
Diphenylamine
o-Aminodiphenyl
α-Naphthylamine
β-Naphthylamine
Pyrrole
Pyridine
2-Aminopyridine
Quinoline
Piperidine[a]
Piperazine[a]
Morpholine
Hexamethylenetetramine

Isocyanates, nitroso compound

Phenyl isocyanate
α-Naphthyl isocyanate
Diphenylnitrosoamine[a]

Nitro compounds

Nitromethane
2-Nitropropane
Nitroglycerine
m-Dinitrobenzene
3,5-Dinitrobenzoic acid

Amino alcohols (alkamines)

Ethanolamine[a]
Diethanolamine[a]
2-Amino-2-methyl propanol
2-Amino-2-methyl-1,3-propanediol
Tris-(hydroxymethylaminomethane)
Triethanolamine[a]
Diisopropanolamine
Triisopropanolamine[a]
o-Aminophenol
m-Aminophenol
Ethylphenylethanolamine
2-Ethanolpyridine
4-Ethanolpyridine

Nitriles

Acetonitrile
Aminoacetonitrile
Acrylonitrile
1,4-Dicyano-2-butene
Methyleneaminoacetonitrile
Adiponitrile
ε-Aminocapronitrile
Sebaconitrile

Oximes, hydroxamic acid

Acetone oxime
Dimethyl glyoxime
Butyraldoxime
Heptaldoxime
Cyclohexanone oxime
Salicylaldoxime
Acethydroxamic acid

Cyanic acid derivatives

Cyanamide
Dicyandiamide
Cyanuric acid
Ammelide
Ammeline
Melamine

Amidine salt

Guanidine nitrate

---

[a] Titrated in the presence of acetic acid; all others used methanol as diluent.

Formaldehyde cyanohydrin analyzed 0.88 ± 0.03 per cent water initially and 1.42 per cent after a calculated additional 0.51 per cent had been added. The oximes of acetone, dimethyl glyoxal, and salicylaldehyde gave values of 0.93 ± 0.02, 0.00 ± 0.00, and 0.20 ± 0.01 per cent water, respectively, in duplicate titrations.

Guanidine nitrate, $NH_2C(=NH)NH_2 \cdot HNO_3$, could be titrated without interference (0.00 per cent water found). Guanidine carbonate, $[NH_2C(=NH)NH_2]_2 \cdot H_2CO_3$, and aminoguanidine carbonate, $NH_2 \cdot NHC(=NH)NH_2 \cdot H_2CO_3$, however, could not be titrated for water alone. In both cases the carbonic acid groups reacted quantitatively with the Karl Fischer reagent, following the inorganic reaction to be described in Chapter VIII. The molar ratios of apparent water found to amidine carbonate added were 1.00 and 1.04 for guanidine carbonate and aminoguanidine carbonate, respectively.[8] Since the interfering reaction was quantitative, the water content of these two compounds could be calculated provided that the total carbonate was determined by an independent method.

The protein, trypsin, was found to contain 8.09 ± 0.04 per cent water in a series of 10 determinations.[6] An unidentified protein reported by McKinney and Hall[31] analyzed 11.1 ± 0.00 per cent water, compared to 10.95 ± 0.15 per cent by chloroform distillation and 10.9 ± 0.00 per cent by oven drying. A sample of cyanamide was found to contain 0.02 ± 0.00 per cent water while melamine gave values of 0.05 ± 0.00 per cent.[8] Naphthyl isocyanate and diphenylnitrosoamine analyzed 0.007 ± 0.001 and 0.086 ± 0.003 per cent, respectively, initially and 0.83 ± 0.01 and 1.62 ± 0.02 per cent after an additional 0.82 and 1.51 per cent water had been added.[8] Analyses on nitromethane gave 0.94 ± 0.02 per cent water by direct titration and 2.05 ± 0.03 after sufficient water had been added to give a total calculated 2.03 per cent.[8] Featherer[45a] compared the Fischer reagent titration with toluene distillation for the estimation of water in two samples of crude nitrobenezene (Nos. 1 and 2 below). In another series of experiments he added known quantities of water, aliquoted from methanol solution, to purified nitrobenzene (obtained by double distillation over calcium carbide) and then titrated the solutions with Fischer reagent (Nos. 3–6). Results on duplicate titrations were:

[45a] Featherer, R. L., Eastern Laboratory, E. I. du Pont de Nemours & Co., Inc., *private communication.*

No.	Water added, wt. %	Water found, wt. %	
		Toluene distillation	Fischer reagent titration
1	0.0	0.13	0.19 ± 0.01
2	0.0	0.24	0.28 ± 0.00
3	0.04		0.065 ± 0.005
4	0.09		0.115 ± 0.005
5	0.21		0.235 ± 0.005
6	0.43		0.47 ± 0.01

(Nitroglycerine and trinitrotoluene will be discussed in Chapter VI under explosives.)

The alkaloids, strychnine and berberine sulfate, analyzed 0.00 per cent water on direct titration and 0.27 ± 0.02 and 0.43 ± 0.01 per cent after a calculated 0.28 per cent water had been added to the former and 0.41 per cent, to the latter.

DETERMINATION OF WATER IN HYDRAZINE DERIVATIVES

Hydrazine sulfate, $(NH_2)_2 \cdot H_2SO_4$, gave no evidence of reaction with Karl Fischer reagent. Duplicate 2 gram samples dispersed in methanol analyzed 0.51 ± 0.01 per cent water initially and 1.32 ± 0.00 after a calculated 1.32 per cent water had been added, including that originally found. However, all hydrochlorides of hydrazine and its derivatives which were studied, when dispersed in methanol and titrated, reacted mole for mole with the iodine of Karl Fischer reagent, possibly according to the reaction postulated by E. Fischer[46] for phenylhydrazine:

$$2\,C_6H_5{\cdot}NHNH_2 + 2\,I_2 \longrightarrow 3\,HI + C_6H_5{\cdot}NH_2{\cdot}HI + C_6H_5{\cdot}\underbrace{NN{=}N}_{} \qquad (2)$$

TABLE XXVI

Reaction of Hydrazine Derivatives with Karl Fischer Reagent[8]

Substance	Amt. added, millimoles	Iodine consumed, millimoles	Ratio
Hydrazine hydrochloride			
$(NH_2)_2{\cdot}2HCl$	8.97	9.20	1.03
Semicarbazide hydrochloride	15.00	16.29	1.08
$H_2NCONHNH_2{\cdot}HCl$	12.15	13.54	1.11
Phenylhydrazine hydrochloride	14.47	14.77	1.02
$C_6H_5{\cdot}NHNH_2{\cdot}HCl$	8.39	8.50	1.01

---

[46] Fischer, E., *Ber.*, 10, 1331–8 (1877).

Analytical data for a few hydrazine derivatives are given in Table XXVI. This reaction appeared to be inhibited when these salts were dissolved in excess acetic acid before titration. Approximately 2 gram samples were dissolved in 25 ml. of glacial acetic acid and titrated with Karl Fischer reagent. Analytical data are recorded in Table XXVII.

TABLE XXVII

Determination of Water in Hydrazine Derivatives[8]

Substance	Initial water found, wt. %	Additional water, wt. %	
		Added[a]	Found
Hydrazine hydrochloride	0.07 ± 0.00	2.17	2.16
Semicarbazide hydrochloride	0.39 ± 0.00	2.52	2.50
Phenylhydrazine hydrochloride	0.37 ± 0.01	2.50	2.53

[a] Including water originally found.

In the presence of excess dichloroacetic acid, and boron trifluoride in acetic acid, results were obtained which are comparable to those recorded in Table XXVII.

H. Determination of Water in Neutral Halides and Sulfur Compounds

Halides, together with hydrocarbons, have been used widely as carriers in distillation methods for the determination of water in other substances. All have had the advantage of limited water solubility while forming heterogeneous azeotropes with this compound. Although the halides have been satisfactory as media in the analysis for water in other substances by distillation methods, the small actual water content of these halogenated compounds could not be determined readily in this way. (This also applies, of course, to hydrocarbons.) Those chemical methods which were applicable to hydrocarbons (section E, this chapter) usually could be used for halides and neutral sulfur compounds.

All types of neutral halides and many sulfur compounds, including sulfides (thio ethers), disulfides, thiocyanates, isothiocyanates, and thio esters, have been titrated for free water without interference from the Fischer reagent. However, the halides and some sulfur compounds, like the hydrocarbons, were poor solvents for the Fischer reagent endproducts and could be titrated only in the presence of methanol or other mutual, inert solvents. Some of the compounds which have been analyzed in the authors' laboratory using the Karl Fischer reagent are[8]:

Halides		Sulfides, disulfides	Thiocyanates, etc.
Methyl chloride Methylene chloride Methylene iodide Chloroform Carbon tetrachloride Iodoform Ethyl iodide Ethylene bromide Ethylidene bromide	Chloroprene 1,4-Dichloro-2-butene Bromobenzene Iodobenzene Benzilidene chloride Dichlorodiphenyl tetrachloroethane (DDT) Chloronaphthalene Bromonaphthalene	Carbon disulfide Allyl sulfide *n*-Butyl sulfide *n*-Butyl disulfide Thiophene Methylene blue	Ethyl thiocyanate Ethylene thiocyanate *n*-Butyl thiocyanate Ethyl isothiocyanate α-Napthylisothiocyanate Ethyl thioacetate Benzothiazole Acetyl thiourea Methyl isothiourea

1. Determination of Moisture in Halides

The determination of moisture in methyl chloride (B.P. —23°C.) illustrated an interesting variation of the Fischer technique involving the direct titration of the halide in methanol solution at —30° to —40°C. Homogeneous solutions and stable endpoints were obtained using the following procedure. A 500 ml. Erlenmeyer flask is fitted with a standard 28/15 spherical grind and Ŧ 14/20 side arm, as shown in Figure 27. The side arm is stoppered and exactly 100 ml. of dry methanol (<0.05 per cent water) are added. A one-holed rubber stopper containing a drying tube is inserted into the neck of the flask, the side tube is stoppered, and the whole is weighed on a torsion balance. The flask is then transferred to a dry ice–methanol bath. An inlet tube, having a Ŧ 14/20 inner member and connected to the methyl chloride cylinder, is seated through the

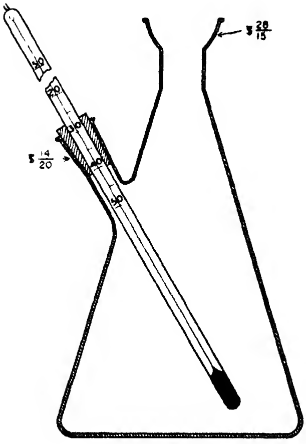


Fig. 27. Flask for Karl Fischer reagent titrations at reduced temperatures using visual endpoint.[8]

side arm of the flask. About a 150 gram sample of the halide is collected in the chilled flask; the side arm is restoppered. The flask is weighed (cold) after which an alcohol thermometer is inserted through the side tube. The flask is connected to the Fischer reagent buret (Fig. 6) and, when the temperature has risen to about —30°C., the Fischer reagent is added until a stable endpoint is reached. (Because the reaction is apparently slower at reduced temperature, the titration is continued until the endpoint is stable for at least 3 minutes.) Results obtained on methyl chloride to which various known quantities of water were added are given in Table XXVIII.

TABLE XXVIII

Determination of Water in Methyl Chloride[8]

Wt. sample, g.	Water, mg.		Net water,[a] wt. %
	Added	Found	
145	0.0	9.3	0.0064
131	33.4	41.6	0.0063
134	122.4	130.7	0.0062
135	119.9	128.9	0.0067
160	0.0	9.3	0.0058
94	90.2	97.0	0.0072
168	123.8	134.7	0.0065
94	121.0	128.2	0.0077
		Average 0.0066 ± 0.0005	

[a] That is, water originally present.

Smaller samples gave consistently higher results with values ranging from 0.0079 to 0.0098 per cent on 69 to 75 grams of the halide. Since the tendency under these conditions was toward slight overtitration, the lower values obtained with the larger samples were considered more reliable. Moisture in methyl chloride also has been determined by means of the apparatus recommended for gases (Fig. 30, Chapter VI). In this unit, another sample of the halide gave values of 0.0015, 0.0018, and 0.0013 per cent (average 0.0015 ± 0.0003 per cent) moisture. Samples to which known water was added analyzed 0.1101 and 0.1140 per cent *versus* 0.1104 and 0.1138 per cent (uncertainty at least ± 0.0003 per cent) calculated, including the moisture found in the original sample.[8] The analysis of monomeric chloroprene, $CH_2{=}C(Cl)CH{=}CH_2$, exemplified the application of the Fischer reagent to extremely active unsaturated compounds. A sample of freshly steam-distilled chloroprene analyzed 0.041 ± 0.00 per cent water and then 0.276 per cent after a calculated 0.280 per cent water had been added, including that originally found.

None of the halides in the above list offered any difficulty in titration, provided that sufficient inert solvent was used. Other investigators have reported that esters, hydrocarbons, and chlorinated hydrocarbons did not dissolve readily in the reagent but that satisfactory results were obtained when these materials were mixed previously with dry methanol.[29]

2. Determination of Water in Sulfur Compounds

a. General

All of the miscellaneous organic sulfur components in the list were titrated directly using methanol as diluent. Quadruplicate analyses of ethyl thioacetate, $CH_3COSC_2H_5$, gave values of 0.39 ± 0.03 per cent water; ethyl isothiocyanate, C_2H_5NCS, analyzed 0.15 ± 0.01 per cent before and 5.32 ± 0.03 per cent after the addition of 5.28 per cent water. (The added water total includes that found in the original sample.) Allyl sulfide, $CH_2{=}CHCH_2SCH_2CH{=}CH_2$, and butyl disulfide, $C_4H_9SSC_4H_9$, analyzed 0.09 ± 0.00 and 0.03 ± 0.005 per cent water, respectively, and, after the addition of 5.22 and 5.20 per cent water, analyzed 5.21 ± 0.02 and 5.20 ± 0.02 per cent. (The added water data include that found in the original sample.) Carbon disulfide and thiophene initially gave zero per cent water and then 0.36 ± 0.02 and 0.34 ± 0.02, respectively, after the addition of 0.35 per cent water to each.

b. Action of Karl Fischer Reagent on Mercaptans

The ease with which mercaptans are oxidized to the disulfide was made the basis of an iodometric method for their determination:[47,48]

$$2\,RSH + I_2 \longrightarrow RSSR + 2\,HI \qquad (3)$$

---

[47] Klason, P., and Carlson, T., *Ibid.*, 39, 738–42 (1906).

[48] Kimball, J. W., Kramer, R. L., and Reid, E. E., *J. Am. Chem. Soc.*, 43, 1199–1200 (1921).

It was not surprising, therefore, to find that mercaptans are oxidized by the iodine of Karl Fischer reagent. Milberger and his coworkers[49] found that ethyl and methyl mercaptans reacted with Fischer reagent in the molar ratio of 2 mercaptans to 1 iodine, as required by equation (3). In a more thorough investigation in the authors' laboratory,[8] the stoichiometry of this reaction for mercaptans in general was studied (see Chapter XV) and a technique by which free water could be determined in the presence of mercaptans was developed. This new method is based on prior addition of the mercaptan to an active unsaturate:

$$\mathrm{RSH} + \mathrm{R'CH{=}CHR'} \xrightarrow{\mathrm{BF_3}} \mathrm{R'CH(SR)CH_2R'} \qquad (4)$$

The addition compound is inert toward Fischer reagent.

Development of New Method for Determination of Water in Presence of Mercaptans. Posner[50] found that thiophenol would react with isopentene to form an addition compound in the presence of acetic and sulfuric acids as catalysts. Although no conditions were given, the reaction seemed to offer some possibility as a means of stabilizing mercaptans in general. In initial experiments 1 ml. quantities (7.2 millimoles) of *n*-hexyl mercaptan were treated with various unsaturates under conditions as shown in Table XXIX.

The octenes used under the conditions of experiments 12–17 gave minimum results, 0.72 ± 0.02 per cent. In order to determine whether this value represented actual water content, the hexyl mercaptan was fractionated at 1 atmosphere pressure and 1 ml. (7.2 millimoles) portions of the fraction boiling at 150.0°C. were used for the analysis, along with 6 ml. (38.5 millimoles) portions of isooctene. The catalysts of experiments 12 and 17, and also 10 ml. of acetic acid plus 10 ml. of the boron trifluoride in acetic acid, after 30 minutes at room temperature, gave results of 0.15 ± 0.03, 0.14 ± 0.02, and 0.15 ± 0.04 per cent water, respectively, which did not seem unreasonable since no protection was provided against the introduction of atmospheric moisture during the distillation.

Attempts to verify this figure by azeotropic distillation with benzene and preferential hydrolysis with acetyl chloride[26] were unsuccessful. No positive values could be obtained even after the addition of a known 0.2 per cent water.

---

[49] Milberger, E. C., Uhrig, K., Becker, H. C., and Levin, H., paper presented before the Analytical and Micro section, 110th meeting of the American Chemical Society, Chicago, Ill., September 10, 1946.

[50] Posner, T., *Ber.*, 38, 646–57 (1905).

TABLE XXIX

Reaction of Hexyl Mercaptan (1 ml., 7.2 mM[a]) with Unsaturates

No.	Unsaturate			Catalyst	Conditions	Net apparent water, mM[a]	Apparent water, wt. %
	Compound	ml.	mM[a]				
1	Caprylene	6.5	41.6	None	10 min. R.T.[c]	2.82	6.0
2	Caprylene	6.5	41.6	25 ml. CH_3COOH	10 min. R.T.	2.58	5.5
3	Caprylene	6.5	41.6	25 ml. CH_3COOH + 10 ml. BF_3 in CH_3COOH[b]	10 min. R.T.	0.90	1.9
4	Dipentene	6.0		Same as 3	10 min. R.T.	1.53	3.2
5	Cyclohexene	3.5		25 ml. CH_3COOH	10 min. R.T.	3.23	6.8
6	Cyclohexene	3.5		25 ml. CH_3COOH + 5 ml. BF_3 in CH_3COOH[b]	10 min. R.T.	2.94	6.2
7	Cyclohexene	3.5		Same as 3	10 min. R.T.	2.75	5.8
8	Isooctene	6	38.5	10 ml. BF_3 in CH_3COOH	10 min. R.T.	2.45	3.4
9	Isooctene	6	38.5	Same as 3	10 min. R.T.	0.78	1.6
10	Mixed octenes	6	38.5	5 ml. CH_3COOH + 10 ml. BF_3 in CH_3COOH[b]	10 min. R.T.	0.39	0.83
11	Mixed octenes	6	38.5	Same as 10	20 min. R.T.	0.36	0.77
12	Mixed octenes	6	38.5	Same as 10	30 min. R.T.	0.33	0.71
13	Mixed octenes	6	38.5	Same as 10	30 min. 60°C.	0.34	0.73[d]
14	Mixed octenes	6	38.5	Same as 10	30 min. 60°C.	0.34	0.72[e]
15	Mixed octenes	6	38.5	Same as 10	60 min. 60°C.	0.33	0.71
16	Isooctene	6	38.5	10 ml. CH_3COOH + 3 ml. BF_3 in $(C_2H_5)_2O$[f]	30 min. R.T.	0.35	0.75
17	Isooctene	6	38.5	5 ml. CH_3COOH + 3 ml. BF_3 in $(C_2H_5)_2O$	30 min. R.T.	0.33	0.71

[a] Millimoles.
[b] 100 grams of BF_3 per liter.
[c] 26 ± 2°C.
[d] Corrected for the addition of 33.1 milligrams of water.
[e] Corrected for the addition of 30.7 milligrams of water.
[f] Commercial preparation (*ca.* 45 per cent BF_3).

Reagents. The catalyst may be either the commercially available boron trifluoride–ethyl ether solution containing about 45 per cent boron trifluoride or a reagent of acetic acid containing approximately 100 grams of boron trifluoride per liter, prepared according to the directions given in Chapter IX. Either isooctene or mixed octenes may be used as the olefin. C.P. glacial acetic acid and pyridine ordinarily are used as received.

Procedure. To a 250 ml. glass-stoppered volumetric flask containing 5 to 10 ml. of acetic acid is added the sample, containing up to 15 millimoles of mercaptan, followed by 10 ml. of the boron trifluoride–acetic acid reagent or 3 ml. of boron trifluoride–ethyl ether reagent and 6 ml. of octene. The flask, together with a blank containing the same volumes of acid, catalyst, and olefin, is stoppered and allowed to stand for 30 minutes at room temperature. At the end of this time, 5 ml. of pyridine are added carefully and the final solution is titrated with Karl Fischer reagent. The net titer between sample and blank is a direct measure of the water content of the sample.

Results obtained on several mercaptans and other thiol-substituted compounds are given in Table XXX.

TABLE XXX

Determination of Water in Mercaptans[8]

Compound	Initial water found, wt. %	Additional water, wt. %			
		Added[a]	Found	Added[a, b]	Found
Ethyl mercaptan	0.70 ± 0.00	6.30	6.35	18.60	18.55
Isopropyl mercaptan	0.90 ± 0.00	6.30	6.25	18.35	18.35
Butyl mercaptan	0.85 ± 0.00	3.05	3.05	17.70	17.60
Isoamyl mercaptan	0.40 ± 0.00	2.85	2.90	16.35	16.30
Hexyl mercaptan[c]	0.15 ± 0.05	1.85	1.85	6.30	6.25
Heptyl mercaptan	0.70 ± 0.00	4.60	4.60		
Benzyl mercaptan	0.25 ± 0.00	2.80	2.80		
Thiophenol	0.25 ± 0.00				
Thio-β-naphthol	0.05 ± 0.00	1.35	1.35		
Thioglycolic acid	3.85 ± 0.00	6.95	7.00		
Ethyl thioglycolate	0.50 ± 0.05				

[a] Total including initial water found.
[b] Separate experiments.
[c] Distilled; all others analyzed as received.

The new method, based on the experimental data of Table XXX, appeared to be quite precise since results usually checked within ± 0.02 per cent. The absolute accuracy could not be determined on the basis of the above experiments since no independent means was found to cross check the results. The fact that after additional water was added the values obtained checked the total calculated water content indicated that the method was reliable. Based on the sample size of 15 millimoles, as little as 0.05 per cent water could be determined. (By employing larger samples with a corresponding increase in amounts of reagents, it should prove possible to find even smaller quantities of water.)

Interfering Substances. The two lower alcohols, methanol and ethanol, interfered with the method. (Alcohols are esterified in the presence of boron trifluoride—acetic acid even at room temperature (see Chapter IX). Therefore, in any sample containing alcoholic hydroxyl the boron trifluoride–ethyl ether catalyst must be used.) This was first observed when an attempt was made to employ methanol containing boron trifluoride (one hundred grams per liter) as catalyst for the reaction of isooctene with *n*-butyl mercaptan. It may be that the methanol was adding preferentially to the double bond, since Fischer reagent titers were obtained which were roughly equivalent to the total mercaptan. (Addition reactions of acrylonitrile with aliphatic alcohols in the presence of alkaline catalysts[51] and in the presence of $BF_3 \cdot HF$[52] have been reported.)

$$(CH_3)_2CH_2CH_2CH_2CH_2CH{=}CH_2 + ROH \longrightarrow (CH_3)_2(CH_2)_4CH(OR)CH_3 \quad (5)$$

It was thought that if sufficient olefin were present (for alcoholic samples) both reactions (4) and (5) could be made to go to completion. The experiments recorded in Table XXXI were carried out on 0.85 gram (7.2 millimoles) of butyl mercaptan analyzing 0.95 per cent water.

Based on the data of Table XXXI, only methanol and ethanol interfered, at least in the concentrations listed. Tertiary alcohols interfered to a considerable extent in the presence of boron trifluoride, apparently because of dehydration. In the presence of 2 ml. of *tert*-butanol, for example, after ½ hour at room temperature, the Fischer reagent titer was equivalent to about 50 per cent water in a 1 ml. sample. Also, the OH group apparently could interfere when present in the same molecule as the thiol group. A sample of β-mercaptoethanol, for example, gave an apparent water content

[51] MacGregor, J. H., and Pugh, C., *J. Chem. Soc.*, **1945**, 535–6.
[52] Lien, A. P., United States Patent 2,399,126 (1946).

TABLE XXXI

Effect of Alcohols on Determination of Water in Butyl Mercaptan[8]

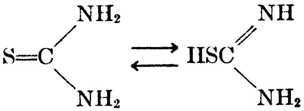
Alcohol, mM[a]		Catalyst, $BF_3(C_2H_5)_2O$, ml.	Unsaturate, mM[a]		Conditions	Apparent water, wt. %
Methanol	50.0	3	Isooctene	38.5	0.5 hr., R.T.[b]	6.3
	50.0	3		38.5	1 hr., R.T.	6.3
	50.0	3		38.5	0.5 hr., 60°C.	3.7
	50.0	3		38.5	1 hr., 60°C.	3.5
	50.0	3		77	1 hr., R.T.	6.3
	50.0	3		77	0.5 hr., 60°C.	3.9
Ethanol	34.8	3		38.5	0.5 hr., R.T.	4.2
n-Propanol	26.0	3		38.5	0.5 hr., R.T.	0.95
n-Butanol	21.0	3		38.5	0.5 hr., R.T.	0.95
	21.0	3		38.5	0.5 hr., 60°C.	0.95
Isobutanol	21.0	3		38.5	0.5 hr., R.T.	0.95
	21.0	3		38.5	0.5 hr., 60°C.	1.0
Methanol	50.0	3	Acrylonitrile	117	0.5 hr., R.T.	5.0
	50.0	3		117	0.5 hr., 60°C.	5.6
	50.0	5		488	0.5 hr., R.T.	4.7
	50.0	10		488	0.5 hr., R.T.	4.3
	50.0	3	Caprylene	51.5	0.5 hr., R.T.	5.1
	25.0	5	Isooctene	77	0.5 hr., R.T.	1.4
	50.0	5		128	0.5 hr., R.T.	6.0

[a] Millimoles.
[b] Room temperature.

of 18.2 per cent. Based on the experience with propyl and higher alcohols, compounds such as δ-mercaptobutanol would not be expected to interfere.

c. Effect of Thiourea and Substituted Thioureas

The titration of thiourea in methanol solution with Fischer reagent was characterized by a slowly fading endpoint, indicative of an oxidation reaction. Assuming the existence of the two tautomeric forms:[53]



thiourea isothiourea

the "iso" or "pseudo" thiourea might be expected to behave as a mercaptan toward reaction with iodine:

---

[53] Richter, V. von, *Organic Chemistry*, Vol. I, Elsevier, New York, 1934, p. 508.

$$2\,HSC({=}NH)NH_2 + I_2 \longrightarrow [H_2NC({=}NH)S]_2 + 2\,HI \qquad (6)$$

This mechanism seemed to offer a reasonable explanation of the interference with Fischer reagent.

In alkaline solution thiourea is oxidized to urea.[54]

$$CS(NH_2)_2 + 4\,I_2 + 5\,H_2O \longrightarrow CO(NH_2)_2 + 8\,HI + H_2SO_4 \qquad (7)$$

$$CS(NH_2)_2 + I_2 + H_2O \longrightarrow CO(NH_2)_2 + 2\,HI + S \qquad (8)$$

Both reactions (7) and (8), however, require the presence of appreciable quantities of water. Furthermore, reaction (7) requires a strongly alkaline environment. S. Skramovsky[54] proposed a quantitative method for the determination of thiourea by reaction with iodine in the presence of 10 per cent potassium hydroxide solution. Equation (8) leads to the formation of finely divided sulfur, whereas the solution remains clear throughout the Fischer reagent titration.

Reaction (6) was supported by the fact that methyl isothiourea, $NH_2C({=}NH)SCH_3$, in which the methyl group is linked to the sulfur, could be titrated without interference. Triplicate 2 gram samples of the C.P. compound dispersed in 25 ml. of methanol gave permanent endpoints when titrated with Fischer reagent, analyzing 0.16 ± 0.01 per cent water. After a total calculated 4.32 per cent water had been added, including that originally present, 4.28 per cent was found.[8]

A sample of finely divided thiourea was exposed over phosphorus pentoxide in a desiccator for several weeks. The anhydrous material, when titrated to the first evidence of an endpoint, gave an apparent moisture content of 2.6 weight per cent, equivalent to the reaction of 0.11 mole of apparent water per mole of compound. Phenylthiourea, $H_2NCSNHC_6H_5$, and diphenylthiourea (thiocarbanilide), $C_6H_5NHCSNHC_6H_5$, behaved similarly. Thiobenzanilide, $C_6H_5CSNHC_6H_5$, apparently also reacted in like fashion. A sample in methanol indicated an apparent water content of about 16 per cent on titration with Fischer reagent. Acetylthiourea, $CH_3CONHCSNH_2$, however, gave no evidence of an interfering reaction; values of 0.09 ± 0.00 per cent water were found.

The thioureas were found to react with the olefin–boron trifluoride reagents used to combine with mercaptans, another indication that combination probably proceeded through the "isothiourea" structure. Duplicate 1 gram samples of thiourea, desiccated over phosphorus pentoxide, were treated with 6 ml. of isooctene and 10 ml. of

---

[54] Skramovsky, S., *Chem. Zentr.*, **1941, II,** 379–80.

boron trifluoride–acetic acid reagent according to the procedure for mercaptans (page 137). Values of 0.02 ± 0.01 per cent water were found. Another sample, analyzed without prior desiccation, gave 0.23 ± 0.02 per cent water initially and then 2.61 per cent after a calculated 2.63 per cent water had been added, including moisture originally found.

This treatment also was effective in inhibiting the interference of thiobenzanilide; a water value of 0.073 ± 0.003 per cent was obtained.

d. Effect of Thioacetic Acid

Thioacetic acid, CH_3COSH, when titrated directly, apparently reacted quantitatively with Fischer reagent, consuming 1 mole of iodine per mole of compound. For example, two samples, each calculated to contain 14.10 millimoles of the thio acid, consumed 14.11 and 14.12 millimoles of iodine, respectively.[8]

Although no dithio acids were examined it was thought that they would react quantitatively because of the apparent ease with which they are oxidized to stable disulfides:[55]

$$2\,RCSSH + I_2 \longrightarrow RCSSSSCR + 2\,HI \qquad (9)$$

Preliminary treatment of thioacetic acid with the olefin–boron fluoride reagent did not indicate any improvement. When an exact equivalent of *n*-butanol was added, however, the mercaptan reagents stabilized the system, permitting titration of the free water in the thioacetic acid.

Presumably the reaction:

$$CH_3COSH + C_4H_9OH \longrightarrow CH_3COOH + C_4H_9SH \qquad (10)$$

takes place. This reaction would be analogous to the hydrolysis of thio acids:

$$RCOSH + H_2O \longrightarrow RCOOH + H_2S \qquad (10a)$$

The butyl mercaptan formed, according to equation (10), is then combined with the olefin (equation 4).

To duplicate 14.1 millimole samples of thioacetic acid, 5 ml. of a dioxane solution containing exactly 14.1 millimoles of *n*-butanol were added, along with 6 ml. of isooctene and 3 ml. of boron fluoride–etherate. (It was essential to add an exact equivalent of

---

[55] Richter, V. von, *Organic Chemistry*, Vol. I, Elsevier, New York, **1934**, p. 320.

n-butyl alcohol, since any excess would react with the acid liberated to form an equivalent amount of water.) After the mixture had been standing at room temperature for 30 minutes, 5 ml. of pyridine were added and the solution was titrated with Karl Fischer reagent. Net water, after correction for the blank titer, was equivalent to 2.25 ± 0.05 per cent. Another sample, to which a calculated 5.62 per cent water had been added, including water originally found, analyzed 5.54 per cent.[8]

The dithio acids probably could be treated in a similar fashion, since they also should interchange:

$$\mathrm{RCSSH} + \mathrm{C_4H_9OH} \longrightarrow \mathrm{RCSOH} + \mathrm{C_4H_9SH} \qquad (11)$$

I. Determination of Water in Organic Peroxides. Effect of Peroxides on Karl Fischer Reagent

1. HYDROPEROXIDES

Hydroperoxides and hydrogen peroxide addition compounds reacted preferentially with the sulfur dioxide of Karl Fischer reagent in a manner analogous to that of hydrogen peroxide (see Chapter VIII):

$$\mathrm{ROOH} + \mathrm{SO_2} \longrightarrow \mathrm{RHSO_4} \qquad (12)$$

Tert-butyl hydroperoxide, $(CH_3)_3COOH$, gave an exothermic reaction apparently according to eq. (12). Since this reaction involved neither water nor iodine, no interference was encountered in the titration for free water. A sample of *tert*-butyl hydroperoxide analyzed 3.60 ± 0.07 per cent water on direct titration with Fischer reagent and 8.95 ± 0.10 per cent after water was added to give a total calculated 9.00 per cent water.[8] Urea peroxide, $H_2NCONH_2{\cdot}H_2O_2$, containing small quantities of water gave a false endpoint on titration with Fischer reagent even in the presence of methanol.

The appearance of a false endpoint in this environment, like that found in systems containing boron trifluoride (see Chapters IX and X), was apparently due to factors other than Fischer reagent endproduct insolubility, since added methanol had no effect. False endpoints are common with some organic substances for which the reaction products are insoluble in the environment used.

When additional water was added, however, the titration was carried directly to a permanent endpoint. Approximately 1 gram samples of urea peroxide, to which 73.3 milligrams of water in methanol solution were added, gave net values of 1.85 ± 0.08 per cent water in triplicate determinations. This peroxide as well as the *tert*-butyl compound gave no evidence of reaction with the hydriodic acid in Fischer reagent. Strongly exothermic reactions occurred when these compounds were titrated. However, when the endpoint was reached, no iodine was formed even though the solution had stood for as long as 1 hour at room temperature.

Pretreatment of urea peroxide with pyridine-sulfur dioxide solution eliminated the false endpoint. Two gram samples were treated with 30 ml. of 1 molar $C_5H_5N{\cdot}SO_2$ in methanol, the flask being cooled in an ice bath during the addition. Titration with Fischer reagent gave values of 1.82 ± 0.07 per cent water, after correction for moisture in the blank,[8] as compared with the net value of 1.85 ± 0.08 per cent water obtained by titration after adding a known amount of moisture.

2. Dialkyl Peroxides

Dialkyl peroxides, in general, are more stable than other types. They do not oxidize hydriodic acid to any appreciable extent in the absence of a catalyst (see Chapter XIV). When titrated with Fischer reagent, neither diethyl nor di-*tert*-butyl peroxide gave any evidence of interference. A 2 ml. sample of diethyl peroxide, $C_2H_5OOC_2H_5$, diluted with 25 ml. of dry methanol and titrated directly with Karl Fischer reagent, gave values of 1.34 ± 0.00 per cent water. After exposure for 24 and 48 hours to Drierite in a desiccator, the peroxide analyzed 0.19 and 0.16 per cent water, respectively. On adding sufficient water to the desiccated material to give 1.78 per cent, including original water found, the peroxide gave results of 1.77 ± 0.01 per cent. Di-*tert*-butyl peroxide, $C_4H_9OOC_4H_9$, on direct titration was found to contain 0.040 ± 0.002 per cent water. After the addition of water to give total calculated values of 0.585 and 1.03 per cent, the sample analyzed 0.584 and 1.06 per cent water, respectively.[8]

3. Diacyl Peroxides

The diacyl peroxides tend to interfere in the Karl Fischer titration for water. Zimmermann[3] reported that benzoyl peroxide did not behave in the same manner as hydrogen peroxide but reacted with the hydrogen iodide present in Fischer reagent, liberating free iodine and forming benzoic acid according to the equation:

$$(C_6H_5COO)_2 + 2\,HI \longrightarrow 2\,C_6H_5COOH + I_2 \qquad (13)$$

However, he gave no indication of the rate of reaction or extent of the interference in the titration for free water. Obviously a reaction of this type would result in low apparent water recovery. Actually for most purposes the interference by benzoyl peroxide was negligible. To individual 2.5 gram samples of pure anhydrous benzoyl peroxide (analyzing 0.00 per cent water by Fischer reagent titration and 99.9 per cent peroxide by reaction with hydriodic acid—see page 145) were added 80.0, 90.0, 160.0, and 307.5 milligrams of water. Fifty milliliters of dry methanol were added and the mixture was titrated rapidly with Fischer reagent. Respective quantities of water found were 79.8, 89.6, 159.4, and 306.3 milligrams.[8] Similar recoveries were observed in the presence of lauroyl peroxide, $(C_{11}H_{23}COO)_2$, originally analyzing 0.00 per cent water. After a calculated 90 milligrams of water were added, a net of 90.3 milligrams were found.[8]

With the more active lower diacyl peroxides the interference became more pronounced. Approximately 1 gram samples of 30 per cent acetyl peroxide, $(CH_3COO)_2$, in dimethyl phthalate solution were titrated with Fischer reagent. The effects of temperature on the titration are shown in Table XXXII. The data indicated that the oxidation of hydriodic acid by the peroxide was fairly slow, since the apparent water content decreased as larger quantities of water were added. The maximum value (1.42 per cent water) was obtained at —40°C., but even at this temperature the net water found was dependent on the quantity of water present. Succinyl peroxide, $O_2(COCH_2CH_2COOH)_2$, behaved similarly.

The approximate moisture content of these active peroxides could be determined indirectly. Apparently they were reduced completely in the Fischer reagent environment on standing up to 30 minutes at room temperature (see Chapter XIV). Therefore, "pre-titrated" Karl Fischer reagent* was added to a sample of the peroxide and the solution was allowed to stand for at least 30 minutes at room temperature. The net millimoles of iodine formed

---

* These experiments were made using Fischer reagent prepared about 1 month previously. In this aged solution the rate of parasitic side reactions was at a minimum (see Chapter III).

TABLE XXXII[8]

Titration of Acetyl Peroxide in Dimethyl Phthalate with Karl Fischer Reagent[a]

Temperature, ° C.	Water, mg.			Net water, wt. %
	Added	Found	Net	
26	0.0	11.7	11.7	0.95
28	0.0	10.1	10.1	0.85
28	16.6	21.0	4.4	0.35
0	0.0	11.6	11.6	0.95
0	16.6	25.6	9.0	0.95
0	79.8	88.2	8.4	0.90
−20	4.4	20.1	15.7	1.20
−20	79.8	95.8	16.0	1.15
−20	154.8	164.2	9.4	0.85
−40	4.4	18.7	14.3	1.40
−40	79.8	93.5	13.7	1.20
−40	154.8	165.8	11.0	1.00

[a] Samples contained about 300 milligrams of peroxide.

was determined by titration with standard water-in-methanol. Subtracting the millimoles of iodine formed from the total millimoles of peroxide added (as determined by an independent analysis) gave the water content of the sample. For example, 1.1855 grams of 29.8 per cent acetyl peroxide (2.99 millimoles) in dimethyl phthalate* were added to a 250 ml. glass-stoppered volumetric flask containing spent Karl Fischer reagent obtained by titrating 10 ml. of a methanol solution containing about 150 milligrams of water to the endpoint. The flask was stoppered and allowed to stand for 30 minutes at room temperature. The total iodine formed was determined by titration with standard water-in-methanol (2.8 milligrams of water per milliliter) to the disappearance of the iodine color. Only 1.52 millimoles of iodine were found. The net millimoles between

---

* The actual peroxide content was determined by a technique developed in this laboratory for the analysis of peroxygen compounds.[8] One gram of granulated potassium iodide is added to a 150 ml. Ŧ 10/10 two-neck, pear-shaped flask. A small straight condenser (with a side arm at the top) is attached and the system is flushed through the flask sidearm with oxygen-free nitrogen. Then the sample, containing up to 2 millimoles of peroxide, 20 ml. of glacial acetic acid, and 15 ml. of chloroform or carbon tetrachloride, is added. The nitrogen line is transferred to the side arm of the condensor to maintain an inert atmosphere while the contents of the flask are gently refluxed for 30 minutes. At the end of this time, 100 ml. of water are added and the iodine in the mixture is titrated with 0.1 *N* sodium thiosulfate to the starch endpoint.

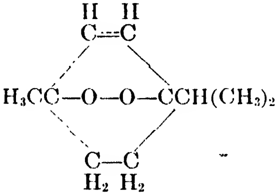
peroxide added and iodine found (2.99 — 1.52 = 147) was equivalent to 2.2 per cent water in the original sample, as compared to a maximum of 1.42 per cent found in direct titration.[8] Succinyl peroxide was treated in the same manner. A sample of 95.9 per cent peroxide (0.0929 gram, 0.38 millimole) gave 0.28 millimole of iodine. The net iodine consumption (0.38 — 0.28 = 0.10) was equivalent to 1.9 per cent water.[8]

No information was available on organic peracids but it is expected that they would behave in a manner similar to that of the active diacyl peroxides.

4. Miscellaneous Peroxides

Triacetone peroxide, $[(CH_3)_2COO]_3$, gave a strong exothermic reaction on titration with Fischer reagent but no iodine was regenerated on standing. The iodine consumption was equivalent to 3.64 ± 0.04 per cent water.[8]

Ascaridole, the terpene peroxide:



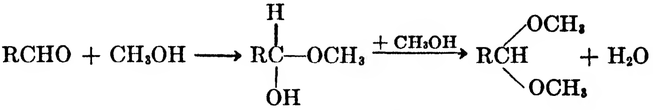
gave no evidence of reaction, analyzing 0.00 per cent water and then 0.86 ± 0.01 per cent after a calculated 0.86 per cent water had been added.[8] This peroxide was extremely stable; it did not respond to the usual methods of analysis for peroxides.

J. Determination of Water in Carbonyl Compounds

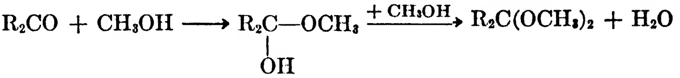
Although modified Karl Fischer reagents or special solvents may be used to inhibit interfering side reactions of many ketones, the cyanohydrin technique is the only general method for the determination of water in the presence of carbonyl compounds.

1. Preliminary Studies

Carbonyl compounds represented one of the few classes of organic compounds which, in general, interfere in the Fischer titration. This was due to their tendency to form acetals or ketals by reaction with the methanol of the standard reagent with the production of additional water:



$$\mathrm{RCHO} + \mathrm{CH_3OH} \longrightarrow \mathrm{R}\overset{\mathrm{H}}{\underset{\mathrm{OH}}{\mathrm{C}}}\mathrm{-OCH_3} \xrightarrow{+\mathrm{CH_3OH}} \mathrm{RCH}\begin{matrix}\diagup\mathrm{OCH_3}\\ \diagdown\mathrm{OCH_3}\end{matrix} + \mathrm{H_2O} \quad (14)$$



$$\mathrm{R_2CO} + \mathrm{CH_3OH} \longrightarrow \mathrm{R_2}\underset{\mathrm{OH}}{\mathrm{C}}\mathrm{-OCH_3} \xrightarrow{+\mathrm{CH_3OH}} \mathrm{R_2C(OCH_3)_2} + \mathrm{H_2O} \quad (15)$$

The presence of carbonyl compounds was characterized by rapidly fading endpoints and erroneously high apparent water contents. The interference was most marked with aldehydes and the lower ketones in whose presence the endpoints faded so rapidly that no more than an approximation of the true water content could be obtained directly.

A series of 5 carbonyl compounds was titrated stepwise with the regular Fischer reagent until a stable endpoint was obtained. After 41 hours at room temperature plus 3.5 hours at 60°C., acetone, butyraldehyde, cyclohexanone, and benzaldehyde had reacted essentially quantitatively and methyl isopropyl ketone, about 70 per cent.

Carbonyl compounds which did not interfere in the standard Karl Fischer titration included such substances as:

Aldehydes	Ketones	
Formaldehyde	Diisopropyl ketone	Benzoin
Chloral	Camphor	Alizarin
Sugars	Benzophenone	Quinalizarin
	Desoxybenzoin	Dibenzalacetone
	Benzil	Triketohydrindene hydrate

By greatly reducing the methanol and increasing the pyridine in the regular Fischer solution, a modified reagent was obtained which reacted normally in the presence of ketones provided that large amounts of the lower alcohols were absent.[4] A reagent consisting of 84.7 grams of iodine in 920 ml. of pyridine and 30 ml. of methanol with 45 ml. of liquid sulfur dioxide* gave a stable endpoint with cyclohexanone (0.02 ± 0.00 per cent water directly and 1.09 ± 0.02 per cent water after an additional 1.06 per cent water had been added), acetone, and methyl isobutyl ketone.

---

* One liter of standard Fischer reagent contains 84.7 grams of iodine, 269 ml. of pyridine, 667 ml. of methanol, and 45 ml. of sulfur dioxide.

Equally satisfactory results were also obtained when the following quantities of pyridine and methanol were used:

Pyridine, ml.	Methanol, ml.
892	60
876	75
860	90

Appreciable quantities of the lower alcohols in the sample permitted the interfering ketal reactions, using the reagent which contained 30 ml. of methanol per liter.

The following data clearly demonstrate the interference of methanol in acetone and the lack of a side reaction of cyclohexanol in cyclohexanone:

Ketone	Amt. of ketone, ml.	Alcohol, ml.	Water found, wt. %
Acetone	5	0.0	0.71
	5	1.0	0.78
	5	2.0	0.81
	5	5.0	0.91
	5	10.0	1.06
Cyclohexanone	5	0.0	0.23
	5	1.0	0.25
	5	5.0	0.24
	5	10.0	0.24
	5	25.0	0.23

With aldehydes, however, consistently low results were obtained when reagents low in methanol and high in pyridine were used.

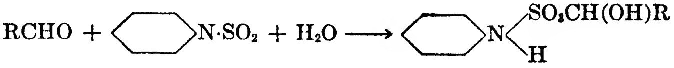
Aldehyde[a]	Amt. of aldehyde, ml.	Water, mg.	
		Added[b]	Found (total)
n-Butyraldehyde	10	0.0	21
	25	0.0	19
	50	0.0	17
	5	50.0	55
	10	100.0	83.6
	15	150.0	93.4
Isovaleraldehyde	10	0.0	44
	10	92.7	111.7

[a] To which known water was added.

[b] Does not include water found in original sample.

Higher recoveries were obtained when relatively small quantities of water or aldehyde were involved.

This suggested the reaction, analogous to that of aldehyde with sodium bisulfite:



(16)

A modified reagent containing excess pyridine apparently was of value only for the direct titration of ketones. Fischer[1] included acetone as one of the materials which could be titrated with his reagent. The indicated high accuracy (0.250 per cent found *versus* 0.249 per cent calculated) was hard to explain unless benzene instead of methanol was used as solvent. The authors found that substitution of dioxane or benzene for methanol gave a stable endpoint with carbonyl compounds but the reagent, as explained in Chapter IV, was decidedly inferior to the alcoholic reagent. Substitution of ethanol for methanol tended to reduce the rate of interfering reactions.[8]

Wernimont and Hopkinson[29] employing a similar reagent high in pyridine (84.7 grams of iodine, 288 ml. of pyridine, 33 ml. of methanol, and 64 grams of sulfur dioxide), were able to determine satisfactorily various quantities of water in 10 milliliters of acetone, by back titrating excess modified Karl Fischer reagent with standard water solution as shown in Table XXXIII. These data obviously showed no trend with varying water concentrations, and the results determined were reasonably accurate. These same authors recommended a reagent of this type for the analysis of mixtures of alcohols containing up to 15 per cent acetone. For higher concentrations of acetone they recommended the acetyl chloride procedure of Smith and Bryant.[26] This hydrolysis technique, like others employing the same type of reaction, was unaffected by high concentrations of ketones. Aldehydes, however, interfered; their action was characterized by a rapidly fading endpoint.[26]

The successful use of a Fischer reagent high in pyridine for the analysis of ketones suggested the possible use of excess pyridine instead of methanol as solvent for the sample. This was tried on 5 milliliter samples of various carbonyl compounds. Comparative results using 25 ml. of methanol or pyridine are illustrated in Table XXXIV.

TABLE XXXIII

Analysis for Water in Presence of 10 Ml. of Acetone[29]

Sample No.	Fischer reagent added, ml.	Standard water solution back titrated, ml.	Standard water solution, net,[a] ml.	Water, mg.		
				Found	Present[b]	Difference
1	5.00	5.00	1.65	8.8	8.6	+0.2
2	5.00	5.10	1.55	8.3	8.6	−0.3
3	5.00	3.00	3.65	19.5	21.7	−2.2
4	5.00	2.90	3.75	20.0	21.4	−1.4
5	10.00	6.10	7.20	38.4	38.1	+0.3
6	10.00	5.50	7.80	41.7	42.2	−0.5
7	15.00	9.55	10.40	55.5	54.2	+1.3
8	15.00	9.05	10.90	58.2	57.0	+1.2
9	15.00	6.45	13.50	72.1	72.1	0.0
10	15.00	6.05	13.90	74.2	77.7	−3.5
11	20.00	7.40	19.20	102.5	106.2	−3.7
12	20.00	4.50	22.10	118.0	117.1	+0.9
13	25.00	5.80	27.45	146.6	148.9	−2.3
14	30.00	8.20	31.70	169.3	168.7	+0.6

[a] (Ml. of Fischer reagent × R) − (ml. standard water solution back titrated). R = 1.330 ml. of standard water solution per ml. of Fischer reagent. The standard water solution contained 5.34 mg. of water per ml.

[b] Sum of water added to that found in 10 ml. of acetone.

TABLE XXXIV

Titration of Carbonyl Compounds in Presence of Methanol and Pyridine (Using Standard Reagent)[8]

Compound	Solvent	Water found, wt. %
Acetone	Methanol (M)	0.65
CH_3COCH_3	Pyridine (P)	0.55
n-Butyraldehyde	M	0.40
C_3H_7CHO	P	0.10
Crotonaldehyde	M	1.20
$CH_3CH{=}CHCHO$	P	0.85
Methyl isopropyl ketone	M	1.15
$CH_3COC_3H_7$	P	1.10
Cyclopentanone	M	0.45
$CH_2CH_2CH_2CH_2CO$	P	0.40
Cyclohexanone	M	0.20
$CH_2(CH_2)_4CO$	P	0.10
Pyruvic acid[a]	M	2.20
$CH_3COCOOH$	P	1.45
Levulinic acid	M	0.55
$CH_3COCH_2CH_2COOH$	P	0.15
Chloral	M	0.05
CCl_3CHO	M + P[b]	0.05

[a] Analyzed 98.7 per cent acid by titration with standard alkali.

[b] When pyridine alone was used there was evidence of a false endpoint. This was eliminated when a 1:1 methanol–pyridine solution was employed.

In all cases where methanol was used, with the exception of chloral, the endpoints faded rapidly, while the endpoints for the experiments in which pyridine was used faded considerably more slowly. Results were based on the first evidence of an endpoint and indicated (with the data of Table XXXVI) that the use of pyridine as solvent permitted the approximate determination of small water contents of carbonyl compounds in general.

2. DIRECT ANALYSIS OF CARBONYL COMPOUNDS FOR WATER

a. General

All types of carbonyl compounds were rendered inert toward the unmodified Karl Fischer reagent by prior reaction with hydrocyanic acid:[56]

$$\mathrm{RCHO + HCN \longrightarrow RCH(OH)CN} \qquad (17a)$$

$$\mathrm{RCOR' + HCN \longrightarrow RR'C(OH)CN} \qquad (17b)$$

The resulting cyanohydrin did not interfere with the titration for water. In the presence of basic catalysts the equilibria in the above reactions usually strongly favored cyanohydrin formation. Those aldehydes and ketones which were less reactive toward hydrogen cyanide also showed little or no tendency to combine with the methanol of Karl Fischer reagent. Consequently, the failure of a few carbonyl compounds to form appreciable amounts of the cyanohydrin was of no consequence, since these materials would not interfere in the normal titration.

The reagent, a solution of hydrogen cyanide in dioxane (or pyridine) together with sodium or potassium cyanide as catalyst, has been found applicable to all types of aldehydes and ketones. The carbonyl compounds given in Table XXXV were reacted with the hydrogen cyanide reagent and then titrated with Fischer reagent to determine the exact initial water content. Larger known amounts of water were then added to separate portions of the carbonyl compounds and the solutions titrated (after hydrocyanic acid treatment) using the titer of the initial carbonyl as a blank. The usual absolute precision of ±0.1 to 0.2 per cent was exceeded in the work reported in this table.

---

[56] Bryant, W. M. D., Mitchell, J., Jr., and Smith, D. M., *J. Am. Chem. Soc.*, 62, 3504–5 (1940).

TABLE XXXV

Analyses for Water in Carbonyl Compounds[56]

Compound	Initial water found, wt. %	Additional water, wt. %					
		Added	Found	Added[b]	Found	Added[b]	Found
Aldehydes							
Acetaldehyde	0.12 ± 0.02	4.25	4.25	13.05	13.00		
Choral	0.03 ± 0.00	1.35	1.35				
n-Butyraldehyde	0.20 ± 0.02	4.10	4.10	13.05	13.05	21.85	21.85
Isobutyraldehyde	0.17 ± 0.02	4.30	4.30	13.06	13.05		
Crotonaldehyde	0.44 ± 0.04	4.25	4.30	13.05	13.00		
2-Ethyl-1-hexanal	0.06 ± 0.03	4.10	4.10	13.00	13.00	21.80	21.85
Citral[a]	0.08 ± 0.00	1.35	1.35				
Benzaldehyde	0.15 ± 0.04	4.15	4.00	13.00	13.00	21.80	21.70
Vanillin[a]	0.05 ± 0.00	3.00	2.95				
Salicylaldehyde	0.20 ± 0.01	4.25	4.25	13.05	13.05		
o-Nitrobenzaldehyde	0.00 ± 0.03	4.25	4.25	13.05	13.00		
Ketones							
Acetone	0.39 ± 0.02	4.10	4.05	13.05	13.05	21.85	21.90
Ethyl isopropyl ketone	0.30 ± 0.06	4.10	4.05	13.00	13.05	21.75	21.85
Diisopropyl ketone	0.10 ± 0.02	4.10	4.05	13.00	13.05	21.75	21.80
Mesityl oxide[a]	1.32 ± 0.04	4.35	4.30				
Acetonylacetone[a]	0.38 ± 0.02	2.05	2.10				
Phorone[a]	0.98 ± 0.02	3.95	3.95				
Cyclohexanone	0.24 ± 0.01	4.15	4.15	13.00	13.00		
Acetophenone	0.23 ± 0.00	4.25	4.25	13.00	13.00	21.75	21.75
Pyruvic acid[a]	1.42 ± 0.02	2.85	2.85				
Levulinic acid[a]	0.16 ± 0.02	1.95	1.95				
Diacetone alcohol[a]	0.98 ± 0.03	3.85	3.90				

[a] Additional data not previously reported in the literature.
[b] Separate experiments.

The original published procedure[56] on which part of the data in Table XXXV were based employed a 2 per cent solution of hydrogen cyanide in pyridine as reagent. This reagent, however, appeared to be relatively unstable, for on standing it tended to darken. (Sample size in the original procedure was limited to no more than 15 millimoles of carbonyl, insuring a 25 per cent excess of hydrogen cyanide and permitting the determination of a minimum of 0.05 per cent water.) It has since been found desirable to use the more stable solution of 5 per cent hydrogen cyanide in pure dioxane, deferring the addition of pyridine until the analysis is made.

b. Reagents

A 5 per cent (by weight) solution of hydrogen cyanide in dioxane is prepared by adding about 70 ml. of liquid hydrocyanic acid (collected in a cold trap at 0–5°C.) to 1 liter of purified anhydrous dioxane* cooled in a slurry of ice in a good hood. After thorough mixing the solution is removed and allowed to warm to room temperature. This solution is stable and relatively safe as an analytical reagent.

The solution may be conveniently prepared and kept in a 1 liter glass-stoppered Pyrex bottle. The reagent should be stored and dispensed in a well-ventilated hood where the succeeding Fischer reagent titration is also carried out.

A 1 per cent solution of sodium cyanide in methanol is prepared by dissolving 1 gram of C.P. dry sodium cyanide in 100 ml. of dry methanol. (The sodium cyanide solution may tend to become slightly cloudy on standing. This does not affect its activity.)

c. Analytical Procedure

Ten milliliters of the 1 per cent sodium cyanide in methanol solution and 20 ml. of the 5 per cent hydrogen cyanide in dioxane are added to a 250 ml. glass-stoppered volumetric flask. The sample, containing no more than 30 millimoles of carbonyl, is added, fol-

---

* The technique of Eigenberger[57] has been found convenient for the purification of technical 1,4-dioxane. One liter of dioxane and 100 ml. of 1 *N* hydrochloric acid are refluxed for 7 hours under nitrogen. Then the solution is shaken with 0.25 pound of potassium hydroxide pellets and, after settling, the upper dioxane layer is decanted into a bottle, 0.25 pound of potassium hydroxide is added, and the mixture is allowed to stand overnight after thorough shaking. To the dioxane, decanted into a boiling flask, about 15 grams of metallic sodium are added, the mixture is refluxed for 3 hours, and is then distilled. The purified dioxane (B.P. 101.2° to 101.4°C., F.P. 11.7°C.) should contain no more than 0.003 per cent water and be free of acetals and peroxides.

[57] Eigenberger, E., *J. prakt. Chem.*, 130, (2), 75–8 (1931).

lowed by 20 ml. of dry C.P. pyridine. The mixture is shaken and allowed to stand for 30 minutes at room temperature after which it is titrated with Karl Fischer reagent. The total titer minus that obtained by titrating a blank (containing 10 ml. of sodium cyanide solution, 20 ml. of hydrogen cyanide solution, and 20 ml. of pyridine) is equivalent to the free water in the sample.

d. Discussion

This procedure permits the determination of as little as 0.02 per cent water in carbonyl compounds (Table XXXV). In those cases where high accuracy is not required for the determination of small quantities of water in aldehydes, the use of excess pyridine alone will suffice provided that the titration is rapid and the first endpoint is taken. Comparative data for analyses respectively employing prior addition of excess pyridine (30 ml. for a 2 ml. sample) and of hydrogen cyanide according to the above procedure are given in Table XXXVI (see also Table XXXIV).

TABLE XXXVI

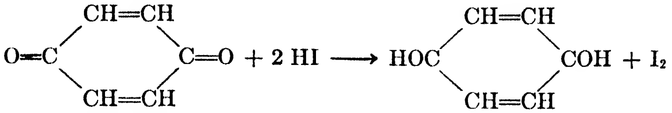
Titration of Aldehydes in Presence of Hydrogen Cyanide or Pyridine[8]

Aldehyde	Reagent	Water found, wt. %
Acetaldehyde	Pyridine	0.24 ± 0.02
	Hydrogen cyanide	0.41 ± 0.01
Isobutyraldehyde	Pyridine	0.20 ± 0.03
	Hydrogen cyanide	0.36 ± 0.01
Crotonaldehyde	Pyridine	0.51 ± 0.03
	Hydrogen cyanide	0.40 ± 0.00
Salicylaldehyde	Pyridine	0.22 ± 0.01
	Hydrogen cyanide	0.20 ± 0.01
o-Nitrobenzaldehyde	Pyridine	0.03 ± 0.03
	Hydrogen cyanide	0.025 ± 0.005

The interfering pyridine–sulfur dioxide–aldehyde reaction [equation (16)] was evident in the analysis of the first two aldehydes where the apparent water found by the use of pyridine alone was actually less than the true water content as given by the cyanohydrin procedure. Suprisingly enough crotonaldehyde appeared to be less active in this respect than the saturated isobutyraldehyde as evidenced by the higher apparent water content for the former when pyridine alone was used. This suggested that acetal formation was favored. Both of the aromatic aldehydes gave accurate values in the presence of pyridiene.

e. Interfering Substances

Quinone probably is reduced by the hydrodic acid always present in Karl Fischer agent:



(18)

liberating iodine at the rate of 1 mole/mole of quinone reacted. Two gram samples in 25 ml. of methanol to which 75 mg. of water had been added gave low recoveries; only 60 milligrams of water were found. When treated with hydrogen cyanide according to the procedure for carbonyl compounds (page 153), the solution became too dark for a visual titration.

K. Determination of Water in Dimethylolurea

Dimethylolurea is formed by the controlled reaction of formalin and urea, probably according to the equation:

$$2\,HOCH_2OH + NH_2CONH_2 \longrightarrow HOCH_2NHCONHCH_2OH + 2\,H_2O \qquad (19)$$

Titrations of solutions containing dimethylolurea with Karl Fischer reagent at room temperature were characterized by an extremely rapidly fading endpoint. A sample containing 91 per cent dimethylolurea by analysis gave apparent water values of from 29.9 to 30.9 per cent in the presence of methanol and also after treatment with hydrogen cyanide according to the carbonyl procedure (page 153). This interference possibly could be due to condensation in which the water released would be continuously removed with Fischer reagent:

$$\begin{aligned}&2\,HOCH_2NHCONHCH_2OH \longrightarrow\\ &HOCH_2NHCONHCH_2OCH_2NHCONHCH_2OH + H_2O \text{ (etc.)}\end{aligned} \qquad (20a)$$

or by condensation with the methanol of Fischer reagent:

$$\begin{aligned}&HOCH_2NHCONHCH_2OH + 2\,CH_3OH \longrightarrow\\ &CH_3OCH_2NHCONHCH_2OCH_3 + 2\,H_2O\end{aligned} \qquad (20b)$$

However, the interfering reaction was so rapid that by the normal titration procedure no estimate of actual water content could be made.

It was observed that on reducing the temperature of the sample, the rate of the interfering reaction was continuously decreased until, at a temperature of —40°C. or below, a permanent endpoint was obtained upon which could be based a quantitative method for the determination of water in dimethylolurea.[8] A sample of about 1 gram is transferred to a flask of the type used for the analysis of methyl chloride (Fig. 27) containing 25 ml. of dry dimethylformamide. The flask is stoppered and shaken until the material is in solution, with warming if necessary. The flask is then connected to the Karl Fischer reagent buret (Fig. 6) and an alcohol thermometer is inserted through the side arm. The contents are cooled to about —40°C., using a dry ice–methanol bath and, while maintaining the low temperature, the solution is titrated slowly to a permanent endpoint. Correction for water in the dimethylformamide is obtained by separate titration of an equal volume under the same conditions. The effect of added water is shown in Table XXXVII.

TABLE XXXVII

Analysis of Dimethylolurea in Dimethylformamide Solution at —40°C.[8]

Sample No.	Initial water, wt. %	Additional water, wt. %[a]			
		Added[b]	Found	Added[b]	Found
1	8.30 ± 0.10	12.3	12.2	14.6	14.7
2	11.90 ± 0.05			15.8	15.5
3	11.35 ± 0.05			15.2	15.5
4	9.10 ± 0.10	13.0	12.8		

[a] Total known water added to individual portions plus initial water found in dimethylolurea.

[b] Separate experiments.

L. Determination of Water of Hydration in Organic Compounds

The use of the Karl Fischer reagent for the direct titration of water of hydration in organic compounds has been discussed for a few compounds but no extensive study has been reported in the literature. However, experiments in the authors' laboratory, which are summarized in Table XXXVIII, have demonstrated the general applicability of the Fischer reagent to the precise and accurate determination of water in these compounds which were titrated in methanol solution or suspension.

Zimmermann,[3] in applying the Fischer method to the analysis of analytical grade oxalic acid, first used an old sample which analyzed 32.35 per cent water, a value in excess of that calculated for the dihydrate (28.59 per cent). An acidimetric check for acid content showed only 67.68 per cent oxalic acid. This same material was finely ground and stored in a desiccator over phosphorus pentoxide. After 36 hours the water content had decreased to 24.56 per cent. When stored over deliquescent calcium chloride, however, the dihydrate was stabilized, as evidenced by the 28.42 per cent water (1.99 moles of water per mole of acid) found for it.

The analysis of citric acid in Table XXXVIII was based on a freshly opened sample. The data reported by Levy and his coworkers[6] for citric acid obviously represented a sample which did not have all of its water of hydration. Their analyses were designed to demonstrate the high precision of the Fischer titration (5.11 ± 0.02 per cent water in a series of 10 determinations).

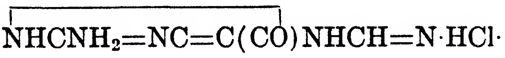
The slightly high value for sulfosalicylic acid dihydrate was shown by Zimmermann[3] to represent additional free water. Based on carbon analysis, the dihydrate calculated 99.82 per cent, permitting 0.18 per cent free water compared to the 0.15 per cent indicated by the Fischer reagent titration.

Sulfanilic acid monohydrate did not appear to be stable at room temperature. A sample recrystallized from water and air-dried initially analyzed 10.98 per cent water, indicating absorbed moisture. After one day in an open beaker at room temperature, 9.90 per cent was found (9.42 per cent calculated for the monohydrate). This value was maintained for a few days before it started to diminish until after 45 days only 7.78 ± 0.08 per cent water remained.[8]

This instability was more marked with cyanuric acid dihydrate which became anhydrous in the course of a few hours.[8] A sample was recrystallized from water, rapidly air-dried, and analyzed for water. After exposure in a small beaker to the atmosphere at room conditions (27 ± 1°C., about 50 per cent relative humidity), approximately 0.6 gram samples were weighed into 250 ml. glass-stoppered volumetric flasks containing 25 ml. of methanol and titrated with Karl Fischer reagent. The results are shown in Figure 28.

It is quite probable that the technique employed for the titration of cyanuric acid, recrystallized from water, can be used to advantage for other systems to demonstrate hydrate formation.

A few other organic hydrates were studied. The so-called guanine hydrochloride dihydrate,



$\text{NHCNH}_2{=}\text{NC}{=}\text{C(CO)NHCH}{=}\text{N}\cdot\text{HCl}\cdot$

TABLE XXXVIII

Determination of Water in Organic Hydrates

No.	Compound[a]	Water found, wt. %	Moles water per mole compound	Reference
	Acids			
1	Oxalic $(COOH)_2\cdot 2H_2O$	28.42 ± 0.04	1.99	3
2	Pyromellitic $C_6H_2(COOH)_4\cdot 2H_2O$	12.71 ± 0.10	2.06	
3	Citric $HOCCOOH(CH_2COOH)_2\cdot H_2O$	9.00 ± 0.10	1.05	
4	*l*-Asparagine $HOOCCH(NH_2)CH_2CONH_2\cdot H_2O$	10.97 ± 0.10	0.91	
5	Creatine $H_2NC(=NH)N(CH_3)CH_2COOH\cdot H_2O$	9.43 ± 0.09	0.91	
6	*l*-Histidine $\underline{NHCH=NCH=C}CH_2CH(NH_2\cdot HCl)COOH\cdot H_2O$	8.78 ± 0.02	1.02	
7	*p*-Toluenesulfonic $CH_3\cdot C_6H_4\cdot SO_3H\cdot H_2O$	9.5 ± 0.10	1.00	
8	*p*-Xylenesulfonic $(CH_3)_2\cdot C_6H_3\cdot SO_3H\cdot 2H_2O$	17.31 ± 0.01	2.13	
9	Naphthalene-β-sulfonic $C_{10}H_7\cdot SO_3H\cdot H_2O$	9.39 ± 0.06	1.18	
10	Sulfosalicylic $2\text{-}HOC_6H_3\text{-}1\text{-}COOH\text{-}5\text{-}SO_3H\cdot 2H_2O$	14.33 ± 0.02	2.02	3
11	Sulfanilic $4\text{-}NH_2C_6H_4\cdot SO_3H\cdot H_2O$	9.90 ± 0.02	1.05	
12	Cyanuric $C_3N_3(OH)_3\cdot 2H_2O$	21.85 ± 0.05	2.00	
	Alcohols, carbohydrates			
13	Terpin hydrate $C_{10}H_{20}O_2\cdot H_2O$	9.42 ± 0.01	0.99	
14	Phloroglucinol $C_6H_3(OH)_3\cdot 2H_2O$	21.70 ± 0.05 23.20 ± 0.10	1.95 2.08	6
15	Dextrose $C_6H_{12}O_6\cdot H_2O$	8.96 ± 0.01	0.99	
16	*l*-Rhamnose $C_6H_{12}O_5\cdot H_2O$	10.03 ± 0.08	1.06	
17	Raffinose $C_{18}H_{32}O_{16}\cdot 5H_2O$	15.49 ± 0.04	5.11	
	Carbonyl compounds			
18	Chloral $CCl_3CH(OH)_2$	10.85 ± 0.02	1.00	
19	Triketohydrindene $C_6H_4\text{-}1,2\text{-}(CO)_2CO\cdot H_2O$	10.0 ± 0.15	0.99	

Table *(continued)*

TABLE XXXVIII (*concluded*)

No.	Compound[a]	Water found, wt. %	Moles water per mole compound	Reference
	Neutral or basic nitrogen compounds			
20	Alloxan $CONHCOCOCONH \cdot H_2O$	11.38 ± 0.12	1.01	6
21	Piperazine $NHCH_2CH_2NHCH_2CH_2 \cdot 6H_2O$	55.3 ± 0.10	5.97	
22	*o*-Phenanthroline $C_{12}H_8N_2 \cdot H_2O$	9.41 ± 0.01	1.04	
23	Methylene blue $C_{16}H_{18}N_3ClS \cdot 3H_2O$	13.3 ± 0.2	2.80	
24	Brucine $C_{23}H_{26}O_4N_2 \cdot 4H_2O$	11.87 ± 0.20	3.08	
25	Brucine sulfate $(C_{23}H_{26}O_4N_2)_2 \cdot H_2SO_4 \cdot 7H_2O$	11.50 ± 0.10	6.50	

[a] Approximately 10 millimoles of water titrated.

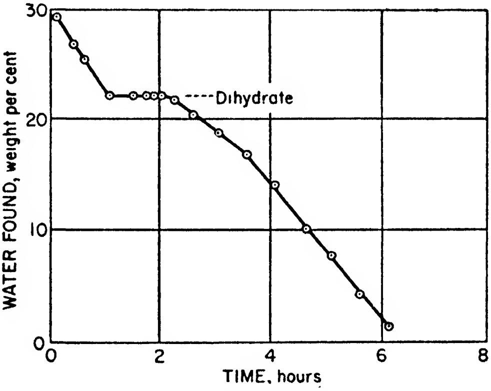


Fig. 28. Titration of cyanuric acid recrystallized from water.[8]

$2H_2O$, was insoluble during direct titration with Fischer reagent; the total titer was equivalent to 30 per cent of that calculated for the dihydrate. Additional samples of the guanine salt, to which excess Fischer reagent was added, were agitated mechanically for ½, 1 and 2 hours at room temperature. The net water found, after back titration with standard water-in methanol, was equivalent, respectively, to 1.00, 1.00 and 0.99 mole of water per mole of salt. The constant results suggested that all of the water was titrated.

Melibiose, $C_{12}H_{22}O_{11} \cdot H_2O$, analyzed 9.85 ± 0.05 per cent water (5.0 per cent calculated) compared to 9.15 weight per cent loss after oven drying at 104°C. Hematoxylin, $C_{16}H_{74}O_6 \cdot 3H_2O$, gave values of 5.89 ± 0.01 per cent water (calculated 15.17 per cent) which compared favorably with the 110°C. oven-dried weight loss figure of 6.0 per cent.

CHAPTER VI

Determination of Water in Commercial Organic Materials

In the preceding chapter the general applicability of the Karl Fischer reagent to almost all familiar types of organic compounds was demonstrated. These were mostly monomeric substances which could be dissolved in some inert solvent prior to analysis. In this chapter it will be shown that among complex commercial materials, those substances rarely caused difficulty (in the accurate and precise titration for water) which were gases, liquids, or solids readily soluble in anhydrous solvents; provided that the component functional groups were known, the methods of Chapter V could be applied with confidence. Ordinarily, the procedure simply required thorough extraction of the liquefied sample (containing up to 200 milligrams of water) with 10 to 100 ml. of dry methanol prior to titration with Fischer reagent; the exact conditions with respect to size of sample and amount of solvent usually could be set according to the convenience of the operator. Also, these commercial materials generally could be analyzed by independent methods to provide checks on the Fischer reagent titration results.

With insoluble substances, particularly those which tended toward thermal decomposition, the situation was quite different. Analyses by the usual methods (including extractions, azeotropic distillations, and oven dryings) tended to be difficult to reproduce, except under strictly standardized conditions, and these conditions were frequently quite different depending on the natures of the samples under investigation. In order to make analyses practical, it was necessary to reduce the samples to small particle sizes, usually by grinding, which introduced the possibility of moisture changes (in sensitive samples at least) according to the humidity. In applying the Karl Fischer reagent to the analysis for moisture in these insoluble materials, the difficulty, of course, was in the preliminary extraction step; as will be evident from the discussion in this chapter, wide differences of conditions have been recommended by different workers for the same substances.

Since, in general, no proved methods existed for exact determination of water in insoluble organics, the goal of most investigators was to define experimental conditions yielding reproducible, precise results which could be checked by an independent, but usually uncertain, procedure. Thus, analyses by the Fischer titration methods have usually been compared to results by oven drying techniques, although the latter might vary greatly depending on the conditions employed. It was usually difficult to tell whether oven drying results were truly quantitative and due to moisture losses alone, or due in part to losses of other volatiles. (Azeotropic distillations, although specific, were often neither sensitive nor precise.)

Titrimetric results varied widely on the low side, depending on conditions for the extraction of moisture. The bulk of evidence seemed to indicate, however, that room temperature extractions with methanol, in general, could be made quantitative, provided that adequate contact of the solvent with the sample could be attained. In other words, the samples should be very finely divided to form a suspension in methanol or other solvent. This condition was approached by use of the Waring Blendor[1] or Hydraetor,[2] both of which are described in this chapter. It is interesting to note that substances such as sugars, cottons, wools, papers, molding powders, explosives, etc., apparently were analyzed quantitatively without drastic reduction of particle size or elevated extraction temperatures. Most fruits, vegetables, and starches, which were relatively delicate thermally, also were relatively difficult to extract quantitatively.

A. Analysis of Petroleum Products for Moisture

1. DETERMINATION OF MOISTURE IN LIQUID HYDROCARBONS

Karl Fischer,[3] using his reagent, made a rather extensive study of the titration of petroleum fractions. He found that in most cases the water could be removed readily from dark oils by extracting twice with anhydrous methanol or some other water-soluble, oil-immis-

---

[1] Morell, S. A., Pabst Brewing Co., Milwaukee, Wisconsin, *private communication*.

[2] Unpublished results from the authors' laboratory.

[3] Fischer, Karl, *Angew. Chem.*, **48**, 394–6 (1935).

cible, inert liquid. The water content of the extract then became a measure of the original water content of the oil.

Clear or lightly colored materials, such as transformer oil, may be titrated directly with remarkable precision using the visual method. Dark oils can be titrated directly only when an electrometric technique is used to ascertain the endpoint.

Fischer's results are given in Table XXXIX (single analyses).

TABLE XXXIX

Visual Titration for Water in Petroleum Fractions[3]

No.	Liquid	Water found, vol. %		Calculated water, vol. %
		Fischer titration	Calcium hydride	
1	Kerosene extract	0.023	0.009[a]	
2	Transformer oil	0.008		
3	Transformer oil shaken twice with anhydrous methanol and the methol titrated	0.006		
4	Cracked benzine[b] (cut 75–100°C.)	0.019	0.007[a]	
5	Cracked benzine + benzene of known water content	0.035	0.027	0.030

[a] Higher values were obtained if the calcium hydride was allowed to react for a long time with vigorous stirring.
[b] Iodine number of this fraction was 132.

Precision to be expected in such analyses is indicated by the following summary of data obtained in the authors' laboratory.[2] Duplicate samples weighing 40 to 50 grams were added to volumetric flasks containing 100 ml. of "pretitrated" methanol. The flasks were stoppered, shaken frequently over a period of 30 minutes, and titrated with Fischer reagent. The endpoints so obtained were permanent even after continued shaking of those samples forming two layers. In all cases the endpoints were easily distinguished; in the two-phase systems, the methanol and oil layers separated immediately, permitting a clear observation of the methanol layer which had extracted the moisture.

Liquid	Water found, ppm.
Kerosene	110 ± 0.0
Mineral oil	50.5 ± 0.5
Petroleum ether (35–60°C.)	122 ± 8.0
Transformer oil (used)	141 ± 4.0

Petroleum ether, the only hydrocarbon in the above series which was completely miscible with the methanol, gave the maximum deviation. Gester's[3a] glycol extraction technique was of value in the extraction of traces of moisture from hydrocarbons (see page 122, Chapter V).

A visual titration method has been submitted to the American Society for Testing Materials for the determination of moisture in oils.[4] In this recommended procedure a solvent containing 50 ml. of benzene and 10 ml. of methanol is employed, which maintains a homogeneous solution when no more than 30 ml. of sample is used. The titration is carried out in a 250 ml. wide-mouth Erlenmeyer flask fitted with a three-hole cork stopper to accommodate a stirrer, the Fischer reagent buret tip, and the sample pipet. During titration the mixture is stirred continuously and the endpoint is observed without removing the flask. The water content of the blank is determined by a separate titration. When samples larger than 30 ml. are used (resulting in two liquid phases) the endpoint may become obscured due to emulsification. Great precautions are observed in handling the sample and titrating under rigidly controlled conditions, including the use of a stopwatch to maintain titration time within narrow limits. This proposed procedure does not appear to be too satisfactory. The fact that conditions must be so carefully controlled indicates either incomplete extraction of moisture from the sample or inadequate protection against the introduction of atmospheric moisture. The proposed solvent is obviously inadequate in maintaining a homogeneous solution in samples of low moisture content; emulsification problems must be increased by the presence of benzene. (Substitution of decalin would probably reduce emulsification troubles; see page 173.)

Since for oils differential titers between blank and sample-plus-blank usually are quite small, the "pretitration" technique using a larger volume of methanol alone would appear to be more desirable. Under these conditions the titer becomes a direct measure of moisture in the sample.

The greater precision of an electrometric method was indicated by the work of Aepli and McCarter[5] who applied the "dead-stop" technique (see page 86, Chapter V) to unleaded gasoline and other petroleum products. They used no inert solvent or extractant, but added the sample (50 to 100 ml.) directly to a protected titration flask and then added 7 to 9 ml. of Karl Fischer reagent. For gasoline, naphthas, and other materials of lower density than Fischer reagent, the electrodes were permitted to extend to the bottom of

---

[3a] Gester, G. C., *Chem. Eng. Progress*, **43**, 17–22 (1947).

[4] Flowers, A. E., procedure submitted to Committee B-9, Am. Soc. Testing Materials, March 23, 1945.

[5] Aepli, O. T., and McCarter, W. S. W., *Ind. Eng. Chem., Anal. Ed.*, **17**, 316–7 (1945).

the flask; for heavier immiscible samples the electrodes were adjusted to contact the upper liquid layer. The mixture was stirred for about 15 minutes and then back titrated with standard water-in-methanol. After each addition of water solution, the mixture was allowed to stir briefly and the two layers were then allowed to separate. This step was apparently necessary in order to obtain a stable galvanometer deflection. On completion of an analysis the sample was withdrawn through a stopcock connection in the bottom of the titration flask. In this way the inner surface of the flask was not exposed to moisture from the atmosphere and, therefore, could be used immediately for the next analysis.

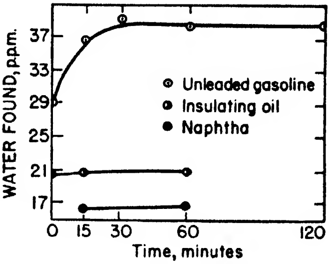


Fig. 29. Effect of stirring time on extraction of water from petroleum products, using excess Karl Fischer reagent.[5]

The time requirement for complete extraction of the moisture was determined on three representative petroleum products which were allowed to contact excess Fischer reagent, with stirring, for periods of 0 to 120 minutes before back titration (Fig. 29).

The data of Aepli and McCarter are summarized in Table XL. In part A, duplicate results are given for various water contents in the three materials of Figure 29, all based on 15 minute extraction periods. Because of the uncertainty of preparing known water-in-hydrocarbon solutions directly, they established the accuracy of their method by employing a third mutually soluble component. Weighed quantities of pyridine containing a known concentration of water were added to exhaustively dried naphtha which then was titrated by their procedure (Table XL, B). The agreement between duplicate determinations was usually within 1 part per million with a maximum deviation of about 2 parts per million and the apparent accuracy (Table XL, B) was within a few tenths of a part per million. However, the accuracy for unleaded gasoline extracted for 15 minutes apparently would be no better than about 2 ppm. (Fig. 29) unless a correction factor was worked out.

Capell, Amero, and Moore[6] used the Fischer titration method in their study of the efficiency of Florite (a commercial desiccant) for the drying of liquid

[6] Capell, R. C., Amero, R. C., and Moore, J. W., *Chem. & Met. Eng.*, 50, 107–10 (1943).

hydrocarbons, such as petroleum fractions ranging from propane to Number 2 fuel oil and including chlorinated hydrocarbons and aromatics. Their reported accuracy of 4 parts per million probably was based on electrometric titration.

Several possible solvent systems were studied by Acker and Frediani[7] in an effort to assure a homogenous environment throughout the Fischer titration. The materials tried included benzene, petroleum ether, naphtha, ethyl ether, methanol, isopropanol, carbon tetrachloride, and tetrachloroethane. A combination of 4 volumes of chloroform plus 1 volume of methanol was found to be the most satisfactory and generally applicable solvent provided that the weight ratio of chloroform to sample was no less than 2:1. In order to minimize the blank titration, chloroform, containing less than 5 ppm. of water, and methanol, analyzing less than 200 ppm. of water, were used. Chloroform of this quality was obtained after periodic agitation of technical grade material with activated silica gel (about 32 g. per kg. of chloroform) over a period of 3 to 4 days.

TABLE XL

Precision and Accuracy of Karl Fischer "Dead-Stop" Method for Determination of Water in Petroleum Fractions[6]

A. Reproducibility of results, ppm. water found

Unleaded gasoline		Naphtha		Insulating oil	
1	*2*	*1*	*2*	*1*	*2*
1.8	1.1	0.5	0.4	5.2	5.6
19.9	18.6	9.1	8.5	8.0	7.4
26.2	27.8	26.2	25.0	9.0	9.7
34.9	34.3	35.2	34.0	28.2	27.3
63.5	62.4	61.2	61.5	31.1	30.2

B. Accuracy of method as applied to naphtha, ppm. water

Present	Found (average)	Present	Found (average)
9.3	8.8	33.6	33.3
20.8	20.5	60.7	60.4
30.7	30.2	104.9	104.4

In making an analysis, Acker and Frediani first added 15 to 20 drops of Karl Fischer reagent (prepared to be equivalent to about 2 milligrams of water per milliliter of reagent) to 125 ml. of the 4 chloroform–1 methanol solution, adjusted the cathode ray tube to the closed position, and then continued the addition of reagent in 0.1 ml. increments until the eye remained open for 30 seconds. (Their direct titration "dead-stop" procedure is mentioned on page 101, Chapter IV.)

A 40 to 60 gram sample of the oil was then added and the titration was repeated using 0.1 ml. portions of the Karl Fischer reagent until the 30 second

---

[7] Acker, M. M., and Frediani, H. A., *Ind. Eng. Chem., Anal. Ed.,* 17, 793–4 (1945).

endpoint was obtained. The second titer was equivalent to the moisture content of the sample.

A series of 30 determinations was made on samples of a light oil, ranging in weight from 40 to 70 grams. Twelve of the results were within 2 ppm. and 8 values deviated by more than 10 ppm. (maximum 25 ppm.) from the average of 44.9 ppm. of water found.[7] Their results on the analysis of transformer oils are given in Table XLI.

TABLE XLI

Determination of Moisture in Transformer Oil[7]

Weight of sample, g.	Water found, ppm.	Deviation from average, ppm.
	New transformer oil	
77	25	−4
56	34	+5
54	31	+2
44	30	+1
50	29	0
52	28	−1
41	27	−2
	Average 29	2
	Used transformer oil	
47	123	+16
40	118	+11
51	98	−9
84	93	−14
79	102	−5
	Average 107	11

Comparison of the direct electrometric technique of Acker and Frediani[7] with the indirect method of Aepli and McCarter[5] from the standpoint of precision of results and instrumental stability indicates the latter to be more satisfactory at the present stage of development. (See also Levy and coworkers[8]; page 99, Chapter IV, gives a description of the apparatus and procedure.)

It has been clearly demonstrated that chloroform may be titrated directly by the visual method or by the back titration electrometric method without any evidence of an unstable endpoint.[2,3,6,9-12] In a private communication,

[8] Levy, G. B., Murtaugh, J. J., and Rosenblatt, M., *Ibid.*, **17**, 193–5 (1945).

[9] Smith, D. M., Bryant, W. M. D., and Mitchell, J., Jr., *J. Am. Chem. Soc.*, **61**, 2407–12 (1939).

[10] Kaufmann, H. P., and Funke, S., *Fette u. Seifen*, **44**, 345–6 (1937).

[11] Kaufmann, H. P., *Olii minerali, olii e grassi, colori e vernici*, **18**, 126, 145 (1938).

[12] Wernimont, G., and Hopkinson, F. J., *Ind. Eng. Chem., Anal. Ed.*, **15**, 272–4 (1943).

Levy reported excellent results for the analysis of a transformer oil containing about 100 ppm. of water, using his microelectrometric method. Hence the instability of the Acker-Frediani direct electrometric endpoint (as evidenced by the relatively poor precision) must have been due either to inadequate protection against atmospheric moisture or to electrical difficulties; their practice of running triplicate determinations and discarding one if it proved to be higher or lower than the other two was further evidence of difficulties with the procedure.

Recent improvements should make better results possible with the direct electrometric titration technique (see page 94ff., Chapter IV.)

Aepli and McCarter[5] pointed out that the Karl Fischer method is not applicable to gasoline containing tetraethyl lead. They found that a sample containing 19.3 ppm. of water gave apparent values of 243 to 247 ppm. after the addition of 4 ml. of Ethyl Fluid per gallon of gasoline. Obviously iodine was consumed by Ethyl Fluid.

The reaction may involve:

$$Pb(C_2H_5)_4 + I_2 \longrightarrow Pb(C_2H_5)_3I + C_2H_5I \qquad (1)$$

This reaction with iodine is the basis of a quantitative method for the determination of tetraethyl lead and other lead tetraalkyls.[13,14]

2. Determination of Moisture in Hydrocarbon Gases

For the estimation of moisture in condensable and noncondensable hydrocarbon gases, Levin, Uhrig, and Roberts[15] employed acetone as the water extractant in conjunction with a modification of the Smith-Bryant[16] acetyl chloride hydrolysis procedure. By substituting methanol or another inert anhydrous solvent for the acetone, the same general technique can be used as a Karl Fischer procedure. In the authors' laboratory[2] a modification of the Levin-Uhrig-Roberts apparatus has been used. This unit, Figure 30, was designed to simplify the method of extraction and to eliminate some of the possible sources of error by reducing the number of evacuations required and permitting the introduction of solvent without removing the flask. Otherwise the procedure followed closely that of Levin and his coworkers.[15]

---

[13] Hein, F., Klein, A., and Mesée, H. J., *Z. anal. chem.*, **115**, 177–83 (1939).

[14] Gunnar, L., *Svenk Kem. Tid.*, **57**, 80–6 (1945); *C.A.*, **40**, 2965 (1945).

[15] Levin, H. L., Uhrig, K., and Roberts, F. M., *Ind. Eng. Chem., Anal. Ed.*, **17**, 212–5 (1945).

[16] Smith, D. M., and Bryant, W. M. D., *J. Am. Chem. Soc.*, **57**, 841–5 (1935).

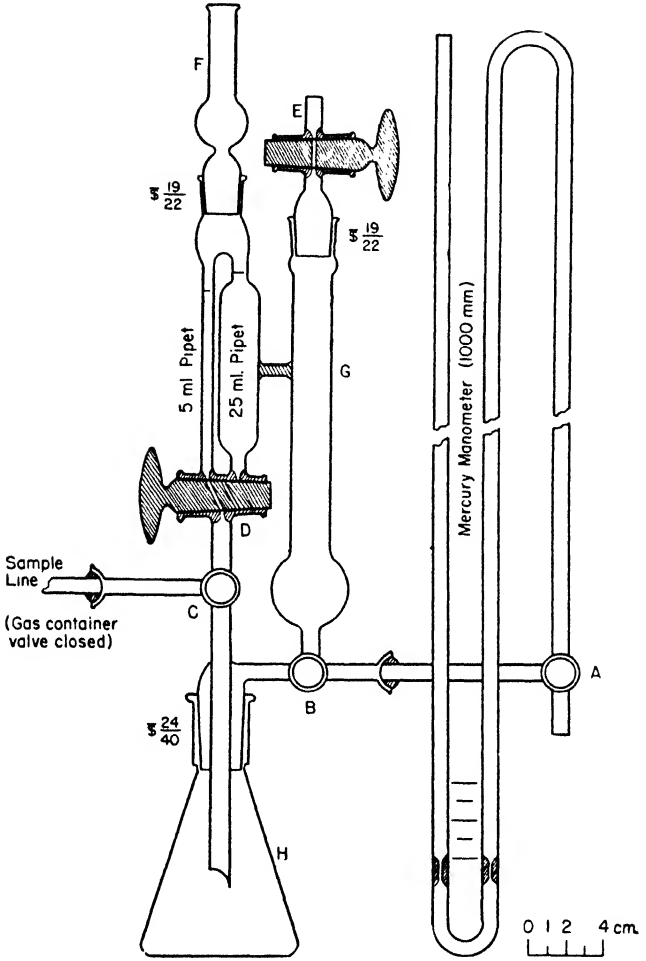


Fig. 30. Apparatus for the determination of moisture in gases.[2]

a. Procedure for Condensable Gases

The apparatus is assembled as shown in Figure 30, except that a standard 19/22 glass stopper is used in place of the desiccant tube F. With stopcock D opened to one pipet, C opened to all three legs, E closed, and B opened to the manometer, the system is evacuated at about 1 mm. of mercury pressure for about 15 minutes to remove moisture. At the end of this time stopcock C is turned to disconnect the sample line and A is closed to cut off the vacuum pump. Then stopcock B is opened to all three legs and E is carefully adjusted to permit the introduction of air through the Drierite-packed drying tube G. When the manometer has indicated a return to atmospheric pressure, stopcock B is turned to disconnect the manometer and D is closed to all legs. Precisely 25 ml. and 5 ml. of dry methanol ($\leq$ 0.03 per cent water) are added to the respective pipets, replacing the stopper with a Drierite tube as quickly as possible. Then the 25 ml. portion of methanol is added to the 300 ml. Erlenmeyer sample flask H.

After sufficient time has been allowed for drainage, stopcock D is closed and a Dewar flask containing Dry Ice-methanol is slowly brought into position around the Erlenmeyer flask. When the temperature of methanol has been lowered sufficiently (after about 10 minutes), stopcock E is closed, B is opened to the manometer, and the system is again evacuated to 1 mm. After closing stopcock A and opening C to the sample line, the sample is introduced and allowed to condense in the Erlenmeyer flask. A maximum of 50 ml. ot sample may be used; the total volume should not be so large as to reach the tip of the delivery tube. Stopcocks C and D are turned to allow the 5 ml. portion of methanol to be drawn slowly into the flask to wash down any moisture which may have frozen in the delivery tube. The cooling bath is lowered and the contents of the flask are agitated for about a minute to insure complete extraction of the moisture into the methanol, after which the bath is replaced. After about 5 minutes, controlled evaporation is begun. Vacuum is applied at A until the manometer indicates subatmospheric pressure. Then the cold bath is lowered and pumping is continued until most of the gas has evaporated, as evidenced by the decrease in volume of the contents of the flask and by the steadily increasing vacuum recorded on the manometer. (The indicated pressure remains essentially that of the vapor pressure of the methanol–gas solution until about 90 per cent of the gas has evaporated; then the manometer will indicate a steadily increasing vacuum.) Stopcock A is closed and a positive pressure of 15 to 25 mm. of mercury is allowed to build up in the system. Stopcock E is opened and B is opened carefully to permit the residual gases to be evolved through tube G. Positive pressure is maintained by manipulation of B during the complete elimination of the gas. At this point the contents of the flask will have returned to room temperature. Then the flask is disconnected from the apparatus and the contents are titrated with Karl Fischer reagent to the visual endpoint (page 74, Chapter IV). A blank is run using 25 ml. and 5 ml. of the same methanol under the same conditions used for samples.

Sample size is best determined by weight loss of the original sample container. If this is not practical, the apparatus may be weighed before and after the addition of sample, after disconnecting the manometer and capping the sample line.

Levin and his coworkers[15] pointed out the potential error due to the small solubility of water in a liquefied hydrocarbon and the large quantity of water possible in the vapor in equilibrium with the liquid phase. Therefore, it was imperative that portions of liquefied samples should not be taken from the vapor side of a container. Where possible, the entire sample should be used, after which the container should be washed with a small volume of the extractant and the washings added to the contents of the Erlenmeyer flask through the tube by which the sample was introduced.

b. Procedure for Noncondensable Gases

For hydrocarbons boiling below the temperature of the Dry Ice-methanol bath (—78°C.), the procedure for condensable gases is followed to the point at which the 25 ml. portion of methanol has been added. The vacuum pump is disconnected and a wet gas meter is connected to the system in its place. The sample is added at a rate not exceeding 30 liters per hour. After sufficient sample has been drawn, the sample inlet tube is washed down with 5 ml. of methanol through C and D, the system is opened to the atmosphere through stopcock E, and the contents of the Erlenmeyer flask are agitated carefully and permitted to return to room temperature. Then the contents of the flask are titrated with Karl Fischer reagent as in the preceding case. About 2 hours are required for a complete analysis.

c. Discussion

The procedure has been used successfully on condensable gases, such as propane, methyl chloride, and dimethyl ether and on one noncondensable gas, air. (Analytical data for dimethyl ether and methyl chloride are given on pages 121 and 132, Chapter V). Approximately 30 gram portions of propane and 30 liters of air were analyzed in the presence and absence of added water. For the latter case a known weight of water was placed in a depression in the sample line inlet which later was allowed to evaporate into the incoming gas sample. Results are given in Table XLII.

TABLE XLII

Determination of Moisture in Propane and Air[2]

Substance	Water		
	Added, mg.	Found, mg.	Found, wt. %
Propane	0.0		0.048
	0.0		0.050
	0.0		0.047
	71.8[a]	71.2	
	36.2[a]	36.7	
Air[b]	0.0	0.8	
	36.7[a]	36.2	

[a] Including moisture originally found.
[b] Passed through Drierite before sampling.

B. Analysis of Oils for Moisture

One of the first reported applications of the Karl Fischer reagent was its use in the determination of moisture in oils and fats.[17] Previously, analyses of these materials were almost exclusively based on azeotropic removal of the water with a hydrocarbon, on oven drying, or on absorption of evolved moisture by a dessicant. A thorough investigation of several methods was made by Joyner and Rini,[18] including the official vacuum oven, air oven, hot plate, xylene distillation, and selective absorption procedures. Of these the last two were the only ones permitting the determination of actual moisture in the oil or fat; the remaining methods did not distinguish between moisture and other volatile matter. The definite need of a reliable method for determination of small amounts of moisture in oils had been pointed out by Parsons and Holmberg[19] who recommended an absorption technique based on evolution of the water at 130–140°C. in an atmosphere of hydrogen and absorption on fused calcium chloride. Joyner and Rini[18] used purified nitrogen as the

---

[17] Richter, *Angew. Chem.*, 48, 776 (1935).
[18] Joyner, N. T., and Rini, S. J., *Oil & Soap*, 16, 233–6 (1939).
[19] Parsons, L. B., and Holmberg, C. D., *Ibid.*, 14, 239–42 (1937).

inert gas and incorporated another absorption tube containing activated carbon ahead of the desiccant tube. The new tube selectively adsorbed the additional volatile matter, usually residual solvent, permitting independent determination of this constituent as well as of moisture. About 2 hours were required for the analyses of a 100 gram sample; average recovery of moisture was 96.9 per cent.

Richter,[17] in a brief article in which no experimental details were given, reported a value of 0.30 per cent for water found in soybean oil by the Karl Fischer titration as compared to 0.29 per cent by xylene distillation. A more thorough study of the application of Fischer reagent titration to analysis of oils was made by Kaufmann and Funke.[10,11] Most fats and oils presented some interference in recognizing the visual endpoint either because of the dark color of the materials or because of precipitation of pyridine hydriodide during direct titration. For these compounds Kaufmann and Funke[10,11] found that an extraction technique was wholly satisfactory, permitting an accurate and precise analysis using visual titration. They dissolved from 20 to 50 grams of sample in about one half the quantity of decalin (decahydronaphthalene) and vigorously shook the solution for some time with 25 ml. of dry methanol in a separatory funnel. After the two clear liquid layers had separated, they discarded the decalin layer and transferred 20 ml. (four-fifths of the original quantity) of the methanol layer to a flask for titration with Karl Fischer reagent. In order to ascertain the blank correction, they treated methanol alone with decalin following the technique employed above. The decalin tended to prevent emulsification which frequently interfered with the clean separation of the methanol layer. Results obtained on several oils, using a single alcohol extraction and titration to a visual endpoint, are recorded in Table XLIII.

Kaufmann found that in all cases tested a single extraction removed all of the water from the decalin solution of the oil or fat. This was proven by carrying out a second extraction in which the methanol layer was found to contain no more water than was found in the blank. A second extraction can always be made when there is some doubt as to the complete removal of water in the first extraction.

Kaufmann indicated that light colored oils which were soluble in the titration liquid, for example, castor oil, were dissolved in methanol and titrated directly, but he gave no experimental data.

TABLE XLIII

Moisture Content of Various Oils Based on Titration of a Separated Methanol Extract[10]

No.	Oil	Water found, wt. %		
		Test 1	Test 2	Test 3
1	Poppy-seed	0.13	0.135	0.14
2	Linseed	0.095	0.09	0.10
3	Rapeseed	0.10	0.095	0.10
4	Peanut	0.075	0.075	0.075
5	Cod-liver	0.165	0.16	0.165
6	Olive	0.095	0.09	0.09
7	Soybean	0.185	0.185	0.18
8	Almond	0.12	0.125	0.125
9	Perilla	0.08	0.08	0.08
10	Pine-seed	0.15	0.16	
11	Sesame	0.055	0.055	
12	Cottonseed	0.10	0.10	
13	Sunflower-seed	0.03	0.03	
14	Neat's-foot 1	0.065	0.06	
15	Neat's-foot 2	0.105	0.11	
16	Maize	0.17	0.18	
17	Sardine	0.11	0.11	
18	Tea-seed	0.09	0.09	

Kaufmann and Funke[20] also demonstrated the applicability of the Smith-Bryant acetyl chloride procedure[16] to the analysis of oils, using the Karl Fischer method as comparison standard. For most oils Kaufmann and Funke, for better solubility, used 10 ml. of 0.2 *M* acetyl chloride in carbon tetrachloride for a 20 to 40 gram sample of the oil, allowing 15 to 30 minutes at room temperature for complete hydrolysis. Then the excess acyl chloride was decomposed with 5 ml. of aniline, followed by titration of total acidity with 0.25 *N* alcoholic potassium hydroxide with phenolphthalein as indicator. Their data are shown in Table XLIV.

The use of decalin, as recommended by Kaufmann and Funke,[10,11] might be helpful for extraction of viscous oils and also of those oils which interfere in Fischer titration (for example, almond oil which contains free aldehyde). In the latter case, separation of the methanol extract would be necessary unless some reagent were added to inhibit the oil's activity (see Chapter V). Otherwise, separation of the methanol extract usually would not be required. Better precision was observed in the authors' laboratory,[2] when the two-phase meth-

---

[20] Kaufmann, H. P., and Funke, S., *Fette u. Seifen,* **44,** 386–90 (1937).

TABLE XLIV

Determination of Water in Oils by Karl Fischer and Acetyl Chloride Procedures[20]

No.	Oil	Water found, wt. %	
		Fischer titration[a]	Acetyl chloride method[a]
1	Soybean	(2) 0.07 ± 0.02	(8) 0.07 ± 0.03
2	Sesame	(1) 0.05	(3) 0.05 ± 0.02
3	Sunflower-seed	(1) 0.10	(3) 0.10 ± 0.02
4	Peanut	(1) 0.10	(3) 0.10 ± 0.01
5	Rapeseed		(5) 0.09 ± 0.04
6	Cod-liver		(3) 0.09 ± 0.03
7	Poppy-seed		(3) 0.13 ± 0.02
8	Castor		(3) 0.18 ± 0.02
9	Sesame		(3) 0.08 ± 0.01
10	Olive		(3) 0.08 ± 0.02
11	Neat's-foot		(3) 0.07 ± 0.01
12	Peach-kernel		(3) 0.10 ± 0.02
13	Cottonseed		(3) 0.04 ± 0.01

[a] Figures in parentheses represent number of individual determinations.

anol–oil system was titrated directly; elimination of the separation step minimized exposure of the methanol to the atmosphere. Duplicate 25 ml. samples of a few oils, including castor oil, were added to 250 ml. glass-stoppered volumetric flasks containing 50 ml. of dry methanol (0.03 per cent water). The flasks were stoppered and allowed to stand for 15 minutes at room temperature with periodic shaking. At the end of this time, the two-phase system (the castor oil was soluble) was titrated with Fischer reagent to the endpoint as given by the first appearance of unused iodine in the methanol layer. In all cases the endpoint was clearly defined and perfectly stable, indicating complete extraction of the moisture from the oil layer. Results, corrected for the water content of the methanol, are given in Table XLV.

An interesting study of the effect of relative humidity on the determination (by extraction) of oil in soybeans was reported by Krober and Collins.[21] Inability to check results was shown to be due to variable humidity conditions, a factor not specified in the official oil extraction technique.[22] In general, the quantity of oil extracted

---

[21] Krober, O. A., and Collins, F. I., *Oil & Soap*, 21, 1–5 (1944).

[22] American Oil Chemists' Society, *Official and Tentative Methods of the American Oil Chemists' Society* (1937).

TABLE XLV

Moisture Content of Oils Based on Direct Titration of Methanol–Oil System[2]

Oil	Water found, wt. %	
	Test 1	Test 2
Rapeseed	0.039	0.042
Peanut	0.022	0.022
Sesame	0.030	0.030
Corn	0.029	0.028
Castor	0.302	0.298
Linseed	0.125	0.129

from the meal was a function of water content—the higher the moisture level the greater the quantity of oil extracted.[23-25] For their experiments, Krober and Collins[21] ground soybeans from several sources in a Wiley mill to pass through a 1 mm. screen and adjusted moisture levels of the meal to 4.35 to 5.00 per cent, 6.40 to 6.90 per cent, and 8.00 to 8.65 per cent. Checks for moisture content, prior to extraction, by the official forced draft method[26] and by the Karl Fischer procedure were within 0.1 per cent.

Krober and Collins used a modified Fischer reagent containing 160 grams (0.63 mole) of iodine and 257 ml. (3.3 moles) of pyridine in 667 ml. of methanol, to which were added 126 grams (1.97 moles) of sulfur dioxide to make 1 liter of solution. Freshly prepared reagent titrated about 6.2 milligrams of water per milliliter. This was nearly double the concentration of iodine and sulfur dioxide, but was approximately the same quantity of pyridine employed by the authors[9] who used 0.33 mole of iodine, 3.3 moles of pyridine, and 1.0 mole of sulfur dioxide with 667 ml. of methanol.

The titrimetric results were obtained with the following procedure. A sample of the meal was transferred to an oven-dried, stoppered Erlenmeyer flask. Fifty milliliters of dry methanol were added and the mixture was shaken, allowed to stand for 1 hour, and then shaken for 15 minutes on a mechanical shaker, after which the mixture was titrated directly with Karl Fischer reagent to a visual endpoint.

---

[23] Bull, W. C., *Oil & Soap,* 20, 94–6 (1943).

[24] Milner, R. T., *Ibid.,* 16, 129–31 (1939).

[25] Taylor, J. J., *J. Assoc. Official Agr. Chemists,* 26, 74–7 (1943).

[26] *United States Department of Agriculture, Agricultural Marketing Service, Service and Regulatory Announcements,* No. 147 (1942).

The analysis for oil content comprised a petroleum ether extraction of the ground meal for 2 hours, a 1 minute regrind, and a second 2 hour extraction. Because of the specific nature of the Fischer reagent these investigators were able to trace the effect of relative humidity on moisture content of the meal, since the petroleum ether did not interfere in the titration. The effect of relative humidity on the moisture content of ground soybeans is shown in Figure 31.

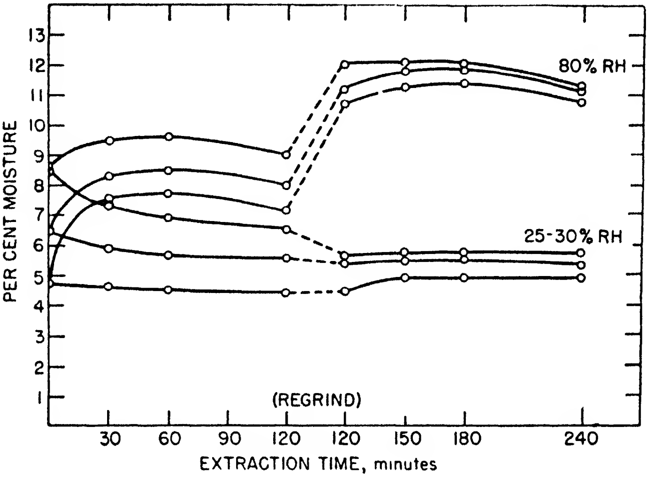


Fig. 31. Effect of high and low relative humidity on moisture content of soybean meal during oil extraction.[21]

Since the quantity of oil which could be extracted from the meal was known to be related to the moisture in the sample, any change in moisture content of the sample during the extraction period or the regrind period might affect the amount of oil extracted under the analytical conditions. The data of Figure 31 clearly demonstrate that relative humidity had a marked effect on moisture concentration in the samples, particularly during the regrind period at high humidity.

In their study of the extraction of oil from meal originally containing 4.73 per cent water and maintained at relative humidities of 25, 50, and 80 per cent, Krober and Collins found that at 80 per cent humidity the amount of oil extracted was 1.2 per cent greater than at 25 per cent humidity.

With samples of meal of original moisture contents varying from 1.5 to 16.8

per cent, but all maintained at about 25 per cent relative humidity, the moisture range was narrowed after the regrind period to about 4 to 6 per cent. One sample, containing 4.7 per cent moisture, remained at a constant level during the entire analysis, indicating that at this level the moisture in the meal was at equilibrium with that in the atmosphere. (This observation was reported to be in agreement with the data of Beckel and Cartter.[27])

Oil extraction at low constant relative humidity was noticeably affected by the original moisture content of the meal, even though the moisture content was reduced during the regrind period. At constant high relative humidity, however, original moisture content exerted much less effect, being noticeable only at the extremes of 1.5 and 16.8 per cent water.

As a result of these experiments, Krober and Collins concluded that with soybean meals of 4.3 to 16.8 per cent initial moisture content, at 75–80 per cent relative humidity, the amount of extractable oil was independent of the original moisture level.[21]

C. Determination of Moisture in Foodstuffs

Vapor pressures were shown to bear a direct relationship to moisture content,[28,29] particularly for food materials relatively high in water. In order to be useful analytically, however, the measurements had to be calibrated against direct methods. This was also true of density measurements for the analysis of meat extracts based on standard dilutions.[30]

Azeotropic distillation methods have been employed successfully. In some cases xylene introduced decomposition errors because of its high boiling temperature. For this reason chloroform, which has a much lower boiling point, has been recommended.[31]

The official method of the Association of Official Agricultural Chemists for the determination of moisture in food materials is based on vacuum-oven drying for a period of 6 hours at a temperature of 70°C. and a pressure of 100 mm. of mercury or less.[32] This procedure is slow and, in general, neither specific nor quantitative, being subject to effects from continuous decompositions.[28,33,34] A number of modifications have been reported, usually for specific materials. For the determination of moisture in whole egg powder,

---

[27] Beckel, A. C., and Cartter, J. L., *Cereal Chemistry,* 22, 362–8 (1943).

[28] Makower, B., and Myers, S., *Proc. Inst. Food Tech.,* 1943, 156–64.

[29] Fischbach, H., *J. Assoc. Official Agr. Chem.,* 28, 186–91 (1945).

[30] Riddle, A. R., *J. Council Sci. Ind. Research,* 18, 153–4 (1945).

[31] Johnston, F. B., *Can. Chem. Process Inds.,* 1943, 100, 102.

[32] Assoc. Official Agr. Chem., *Official and Tentative Methods of Analysis,* 5th Ed., pp. 308, 309, 335 (1940).

[33] Lindsay, W. N., and Mansfield, T., *Ind. Eng. Chem., Anal. Ed.,* 16, 628–30 (1944).

[34] Makower, B., Chastain, S. M., and Nielson, E., *Ind. Eng. Chem.,* 38, 725–31 (1946).

Lindsay and Mansfield[33] reported a rapid procedure employing a pressure of 0.05 mm. of mercury and a boiling water bath to maintain temperature. Samples were dried for 75 minutes after which the vacuum was released through a Dry-Ice trap. Under these conditions the effects of thermal decomposition appeared to be minimized. Makower and his coworkers[34] proposed reference methods for calibration of the official vacuum-oven procedure for dehydrated vegetables. The primary method was based on drying to constant weight *in vacuo* at room temperature over magnesium perchlorate, an inconveniently slow technique. The secondary method was based on oven conditions established by drying samples which had been exhaustively dehydrated (about 100 hours drying time was required) prior to the addition of known water. The latter technique necessarily specified separate conditions for each type of food sample.

In addition to its specificity for water, the Karl Fischer method possesses several advantages over the conventional vacuum-oven procedure. Only 10 minutes to 1 hour are required for a series of analyses by the Fischer method as opposed to 2 to 6 hours by the official method. A single weighing suffices as compared to at least three by the drying procedure.[35]

Richter's[17] brief report on the applicability of the Fischer reagent to the analysis of food materials is summarized in Table XLVI.

TABLE XLVI

Determination of Moisture in Food Materials[17]

No.	Substance	Water found, wt. %	
		Fischer titration	Xylene distillation
1	Cocoa powder	6.0	5.9
2	Wheat flour	11.7	11.8
3	Malt extract	12.3	12.5
4	Margarine	13.0	13.2
5	Marmalade	33.0	32.8
6	Edam cheese	22	29

It is difficult to evaluate the titrimetric procedure on the basis of these data since no experimental details were given. On the basis of the reported results, however, only Edam cheese appeared unsatisfactory for analysis by Richter's modification of the Karl Fischer method.

---

[35] Fosnot R. H., and Haman, R. W., *Cereal Chemistry,* 22, 41–9 (1945).

Kaufmann and Funke[10,11,20] recommended that for butter and margarine about 0.5 to 1 gram of sample be dissolved in 5–10 ml. of chloroform, followed by direct titration. They reported comparative data by three methods as shown in Table XLVII.

TABLE XLVII

Determination of Moisture in Butter and Margarine[20]

Substance	Water found, wt. %		
	Fischer titration	Xylene distillation	Acetyl chloride method
Butter	14.25 ± 0.13	14.3	14.29 ± 0.04
Margarine	11.83	12.1	11.94 ± 0.04

In their study of cereals and cereal products, Fosnot and Haman[35] made a detailed investigation of the Karl Fischer method. The general procedure involved the use of a sample, containing 50 to 150 milligrams of water, which was dispersed in an extraction flask containing 25 ml. of anhydrous methanol (distilled from and stored over Drierite). The mixture in the loosely stoppered flask was brought to the boiling point in a water bath,* allowed to cool, and titrated with Fischer reagent to a 1 or 2 ml. excess. After standing for a specified time, the endpoint was determined electrometrically by back titration with standard water-in-methanol, according to the "dead-stop" procedure (see page 86 ff., Chapter IV).

One of the most critical factors appeared to be that of contact time with the Fischer reagent. Three cereal materials, barley malt, wheat, and corn grits, were ground in a Wiley mill to pass through a 0.5 mm. screen while, for comparative oven analyses by the official A.O.A.C. method, the first two materials were ground in a Seck mill and the corn grits were analyzed as received. (See Krober and Collins' work on soybeans[21] pages 175–178, Chapter VI). The time curves are shown in Figure 32, from which it is evident that the time required to reach maximum moisture values varied with the type of cereal being analyzed. The differences in contact time required evidently were associated with the relative firmness with which moisture was held by the samples.

---

* This step was found to facilitate greatly the dispersion of the sample and complete extraction of water. The small loss of methanol during this treatment apparently had no influence upon the determination, since no water was lost (see page 187).

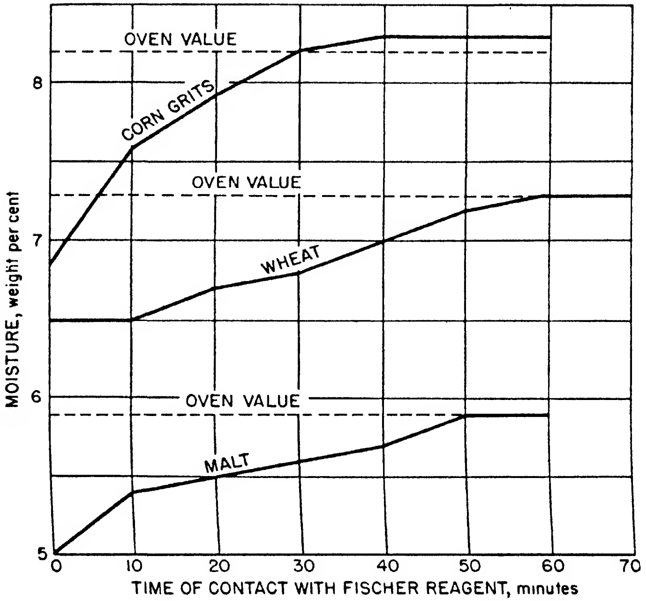


Fig. 32. The influence of time of contact with Fischer reagent on moisture values obtained.[85]

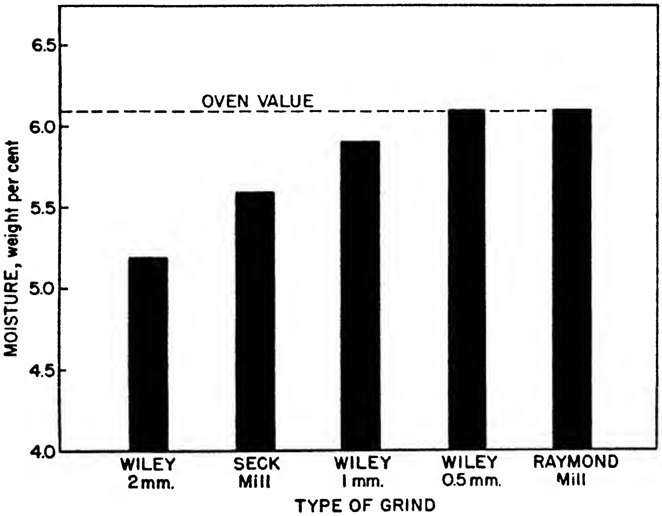


Fig. 33. The influence of particle size of barley malt on the moisture values obtained for 30 minute contact time.[85]

Another important factor, that of particle size, was studied using barley malt. The data presented in Figure 33 were based on a 30 minute contact time. When all other factors were equal, for short contact times, moisture found was related to particle size. (Particle size was equally important in the vacuum oven methods where the moisture must diffuse into the atmosphere.[28,36]) Thus, grinding to 0.5 mm. or less was found necessary for the contact time allotted Barley malt ground with the Seck mill (Fig. 33) gave the maximum moisture value after a 1 hour contact period.

In order to check the precision of the Fischer titration, Fosnot and Haman[35] conducted a series of 10 check analyses each on barley malt and corn starch, both ground to 0.5 mm. or less. The results are shown in Table XLVIII.

TABLE XLVIII

Statistical Summary of Moisture Determinations on Barley Malt and Corn Starch[35]

Statistical value	Moisture found, wt. %	
	Barley malt	Corn starch
Maximum[a]	6.05	12.32
Minimum[a]	5.98	12.14
Mean[a]	5.99	12.22
Oven mean[b]	6.00	11.60
Standard error (single determination)[c]	0.03	0.07

[a] Fischer titration procedure.
[b] Average of duplicate analyses.
[c] Standard error of a single Fischer titration result compared to oven weight loss.

Oven values were based on weight losses in a standard convection oven by the A.O.A.C. methods: barley malt—5 grams of sample, atmospheric pressure, 104°C., 3 hours; corn starch—1 gram of sample, atmospheric pressure, 100°C., 5 hours. The standard errors of 0.03 and 0.07 per cent obtained by their general Karl Fischer method compared favorably with those obtained with other methods. The higher values obtained on corn starch by the Fischer titration probably were in agreement with the results of Sair and Fetzer[37] who apparently demonstrated that the A.O.A.C. procedure did not completely remove the moisture present. With the vacuum oven at

[36] Fischbach, H., *J. Assoc. Official Agr. Chem.*, **28**, 194–7 (1945).
[37] Sair, L., and Fetzer, W. R., *Ind. Eng. Chem., Anal. Ed.*, **14**, 843–5 (1942).

100°C. for 20 hours, they reported an increase of about 1 per cent over the official value.

As an illustration of the versatility of the Fischer method, analyses were made of a wide variety of food materials, using their general technique of 0.5 mm. particle size and 30 minute standing time for solid samples.[35] These data are shown in Table XLIX. The moisture values, ranging from about 1 to 70 per cent, in most cases checked within 0.2 per cent by the two methods. It seems apparent that further study should be made of the relatively wide differences of results by the two methods for corn starch (Table XLVIII) and for wheat flour (Table XLIX). Malt syrup was apparently rather difficult to titrate directly by the Fischer technique. Fosnot and

TABLE XLIX

Miscellaneous Moisture Determinations on Food Materials[35]

Material	Moisture found, wt. %	
	Fischer titration	Oven drying
Spent grain	8.3	8.0
Spent grain	2.9	3.0
Corn flakes	9.35 ± 0.05	9.65 ± 0.05
Wheat flour	12.1	11.4 (5 hrs.) 11.6 (8 hrs.)
Oat flour	8.1	8.1
Soy flour	6.0	5.9
Wheat gluten	7.6	7.5
Wheat	7.1	7.0
Barley	7.3	7.3
Apple nuggets	1.2	1.2
Apple nuggets	1.5	1.5
Apple nuggets	1.7	1.9
Apple nuggets	1.6	1.6
Skim milk powder	4.8 ± 0.0	4.9 ± 0.0
Dried eggs	3.5 ± 0.0	3.7 ± 0.0
Cocoa	2.6	2.7
Malted milk	4.0 ± 0.0	4.1 ± 0.0
Bouillon powder	2.8 ± 0.0	2.4 ± 0.0
Malt syrup	20.0	20.1[a]
Malt syrup	19.6	19.5[a]
Malt syrup	21.5	21.4[a]
Condensed whole milk	62.0	62.3
Condensed skim milk	73.5	73.3

[a] Value by pyknometer.

Haman[35] concluded that sample dilution with water to provide adequate dispersion, with consequent decrease in sample size, reduced the precision and accuracy to a point where the method possessed no marked advantage for routine application.

This difficulty would be eliminated if a suitable solvent or better dispersing agent than methanol were used, possibly acetic acid or glycol. Dispersion of the malt syrup using a Waring Blendor[1] or Hydractor[2] might prove beneficial.

A study of the determination of moisture in ground cottonseed and cottonseed products by the Karl Fischer titration and oven-drying procedures was made by Hoffpauir and Petty.[38] For the direct Fischer reagent analysis, duplicate 1 gram samples were weighed into stoppered flasks, covered with 25 ml. portions of dry methanol, and allowed to stand for 3 hours at room temperature, with frequent shaking, before titration. After 5 gram samples had been oven dried (see Table L for conditions), they were covered with methanol and extracted as above, then titrated by the volumetric procedure. Results are given in Table L.

Hoffpauir and Petty[38] concluded that the high oven weight loss values at 130°C. might be due to decomposition and the lower Karl Fischer values, to incomplete extraction. Since the oven drying and distillation methods were empirical, they were unable to establish the true moisture content by independent means.

Heinemann[39] studied the application of the Karl Fischer titration in the determination of moisture in dairy products, using the glass–platinum electrode system of Lykken and Tuemmler[40] for both the direct and back titration potentiometric techniques. The samples, containing about 100 milligrams of water, were weighed into 100 ml. beakers, 20.0 ml. of dry methanol were added, and the solutions were titrated immediately. (Apparently the titrations were made in open beakers, contrary to the recommendations of previous authors.) In initial studies, results on dry milk solids (3 to 4 per cent water) were obtained both by direct titration with Fischer reagent and by back titration of excess reagent with standard water-in-methanol. Comparative data were obtained by toluene distilla-

---

[38] Hoffpauir, C. L., and Petty, O. H., *Oil & Soap,* 23, 285–8 (1946).

[39] Heinemann, B., *J. Dairy Sci.,* 28, 845–51 (1945).

[40] Lykken, L., and Tuemmler, F. D., *Ind. Eng. Chem., Anal. Ed.,* 14, 67–9 (1942).

tion. Water found by direct and back titration averaged 0.01 and 0.15 per cent higher, respectively, than that found by the toluene procedure.

TABLE L

Comparison of Moisture Found in Cottonseed by Selected Oven Methods and by Karl Fischer Reagent Titration[38]

Material	Water found by Fischer titration, wt. %	Oven drying data		Water found in oven-dried material by Fischer titration, wt. %
		Condition[b,c]	Loss, wt. %	
Fumed and ground cottonseed	5.54[a]	(1) 101°C., F.D., 3 hrs.	5.81	0.45
		(2) 101°C., F.D., 5 hrs.	5.99	0.01
		(3) 101°C., Vac., 3 hrs.	5.60	0.29
		(4) 101°C., Vac., 5 hrs.	5.85	0.02
		(5) 130°C., F.D., 1.5 h.	6.26	0.19
		(6) 130°C., F.D., 3 hrs.	6.40	0.01
Ground cottonseed meats	6.11[a]	(1)	5.99	0.33
		(2)	6.15	0.03
		(3)	5.77	0.13
		(4)	5.96	0.05
		(5)	6.35	0.12
		(6)	6.44	0.03
Cottonseed meal	7.27[a]	(1)	7.40	0.41
		(2)	7.52	0.07
		(3)	7.11	0.12
		(4)	7.39	0.04
		(5)	8.00	0.13
		(6)	8.07	0.00

[a] Average of closely agreeing duplicate determinations.
[b] F.D. is forced draft.
[c] Vac. is vacuum at 100 mm.

Heinemann[39] attributed the higher values found by the Fischer procedure either to the instability of the reagent or to absorption of moisture from the air. The latter alternative appears more likely. Parasitic side reactions which affect the stability of Karl Fischer reagent would be negligible during the course of several successive determinations, assuming that the recommended 24 to 48 hour aging period was observed (see Chapter III). The significantly higher values obtained by the back titration over the direct method strongly indicate that moisture was absorbed from the atmosphere, since the former required more time to complete an analysis and permitted exposure of active Fischer reagent to the atmosphere.

Several comparative analyses were made on dry milk solids by the back titration and toluene distillation procedures. In 20 duplicate analyses of spray-dried nonfat solids, ranging from 2.6 to 4.1

per cent moisture, one set averaged 0.05 per cent lower than the toluene figure, while the others ranged from 0.04 to 0.26 per cent higher. Similar results were observed on nonfat solids which were roller dried and on whole milk powder.

TABLE LI

Determination of Moisture and Total Solids in Dairy Products[39]

Substance	Mojonnier method[a]		Fischer titration[a]	
	Total solids	Water found	Total solids[b]	Water found
Evaporated milk	26.0 ± 0.0	74.0	25.7	74.3 ± 2.7
	25.7 ± 0.5	74.3	27.0	73.0 ± 1.5
Skim condensed milk	30.0 ± 0.1	70.0	27.7	72.3 ± 1.3
Sweetened condensed milk	29.0 ± 0.1	71.0	18.2	71.8 ± 0.3
Whole milk	13.2 ± 0.0	86.8	12.5	87.5 ± 1.2
Butter	83.5 ± 0.0	16.5	83.4	16.6 ± 0.1
	84.1 ± 0.0	15.9	84.1	15.9 ± 0.1
Butter oil	99.85 ± 0.00	0.15	99.85	0.15 ± 0.005
	99.91 ± 0.00	0.09	99.91	0.09 ± 0.00

[a] All results in weight per cents.
[b] Calculated by difference from 100 per cent.

Comparative data for total solids on other dairy products by the Mojonnier[41] extraction technique and volumetric methods (by difference) are given in Table LI. On the basis of the data in Table LI, Heinemann[39] concluded that the Karl Fischer method was unsatisfactory for dairy products containing more than 20 per cent moisture, explaining this conclusion on the basis of the small sample size permitted, errors in the determination of the endpoint, interfering substances such as vitamin C, and instability of the reagent.

The data of Fosnot and Haman[35] (Table XLIX) on the analysis of condensed whole and skim milk indicate no difficulty in the use of the Fischer reagent; comparative analyses were 0.3 per cent less and 0.2 per cent more, respectively, than those obtained by oven drying. Only single analyses were reported, however. An indication of the precision to be expected in the determination of the water in whole and condensed milk was obtained in the authors' laboratory.[2] Samples containing from 200 to 250 milligrams of water were weighed into 250 ml. glass-stoppered volumetric flasks. Twenty-five milliliters of dry methanol were added and the mixtures were titrated immediately with Karl Fischer reagent to the visual endpoint. Triplicate analyses gave results of 88.2 ± 0.2 and 71.0 ± 0.1 per

---

[41] Mojonnier, T., and Troy, H. C., *Technical Control of Dairy Products*. Mojonnier Brothers, Chicago, 1945.

cent on whole and sweetened condensed milk, respectively.

Johnson[42] stored samples of four vegetables in closed containers for at least one month and then ground them in a food chopper. A portion of this material was placed in a closed container while the remainder was rapidly separated into three groups of different average particle size by passage through U.S. Standard sieves. Finally, a part of each of the four sizes was reground through a 40-mesh screen using a Wiley mill. Apparent moisture values were obtained on each subsample as follows. Weighed samples, containing 30 to 200 milligrams of water, were transferred into dry 250 ml. glass-stoppered Erlenmeyer flasks and 25 ml. of dry methanol were added to each flask. (Pyridine and pyridine–methanol were also tried but showed no advantages over methanol alone.) The flasks were connected to a condenser, protected from atmospheric moisture with Drierite, and heated to 60°C. in a glycerol bath. The alcohol was not permitted to reflux because of occasional loss of sample and alcohol by bumping, apparently due to the solids; (see also page 180, Chapter VI). They were then maintained either at 60°C. for 6 hours or quickly removed, stoppered, and placed in an air oven at 59 ± 1°C. for 6 hours. At the end of this period, the moisture was determined by titration with Karl Fischer reagent. Comparative results obtained on samples of varying particle size are given in Table LII. (Allowing the samples to stand in the presence of Fischer reagent at room temperature did not increase the rate of removal of water over methanol extraction alone, and at 60°C. erratic results were obtained.) The results of Table LII seemed to show that, in contrast to cereals (page 181), particle size was an important factor only with white potatoes.

In the oven drying method, the apparent moisture content of carrots varied widely with particle size.[28] Obviously, unless an extremely fine state of division is necessary in order to permit complete moisture extractions, it is safer to minimize grinding because of the possibility of moisture changes during the operation (see Fig. 31).

The effects of time and temperature on the recovery of moisture by methanol extraction were studied and compared with weight loss values by the vacuum–oven method. These data are shown in Table LIII and Figure 34. The dehydrated vegetables were ground in a Wiley mill fitted with a 40-mesh screen.

---

[42] Johnson, C. M., *Ind. Eng. Chem., Anal. Ed.,* **17,** 312–6 (1945).

TABLE LII

Effect of Particle Size on Apparent Moisture Content of Dehydrated Vegetables by Titration with Fischer Reagent[42]

Material	Particle size distribution	Water found, wt. %	
		Material as described in column 2	Material reground to pass 40-mesh sieve
Potatoes (white)	From food chopper (1)	4.97	6.74
	4–10 mesh[a] (2)	3.42	6.82
	10–18 mesh (3)	4.85	6.86
	18–35 mesh (4)	6.32	7.00
Carrots	(1)	7.05	7.20
	(2)	7.18	7.30
	(3)	7.05	7.17
	(4)	7.07	7.06
Cabbages	(1)	6.47	6.54
	(2)	6.48	6.53
	(3)	6.53	6.49
	(4)	6.61	6.60
Onions	(1)	3.84	3.87
	(2)	3.75	3.71
	(3)	3.70	3.73
	(4)	3.72	3.77

[a] Sample passing No. 4, but retained on No. 10, fine series sieve.

According to Table LIII, even when ground to 40 mesh, all samples except carrots showed differences between 4, 6, and 24 hour samples. If not ground, the differences might have been erratic. The data of Table LII are all for 6 hours contact time and seem to be self-consistent. However, corresponding 4 to 24 hour data might have given results indicating different percentages of water depending on particle size. Since the actual moisture content was not known, it would not be safe to assume, on the basis of the 6 hour figures of Table LII alone, that particle size was not a factor in the determination of moisture in cabbages and onions.

There is no accepted procedure at present which permits the specific determination of moisture in dehydrated vegetables. The vacuum-oven method, for example, is unreliable because the continued loss in weight is due in part to loss of actual water from the interior of the cellular material and in part to thermal decomposition.[28,43]

Johnson arbitrarily chose the 38 hour weight loss value (Table LIII) for purposes of comparison. Of the materials studied only peas had reached the flat portion of the curve at this time interval.

---

[43] Porter, W. L., and Willits, C. D., *J. Assoc. Official Agr. Chem.*, 27, 179–94 (1944).

In some cases the Karl Fischer values appeared to be reliable. Carrots showed no increase in apparent moisture for periods up to 24 hours in contact with methanol at 60°C. It was interesting to note that the moisture value so obtained was about 0.5 per cent higher than that found by weight loss after 75 hours at 70°C. (Fig. 34).

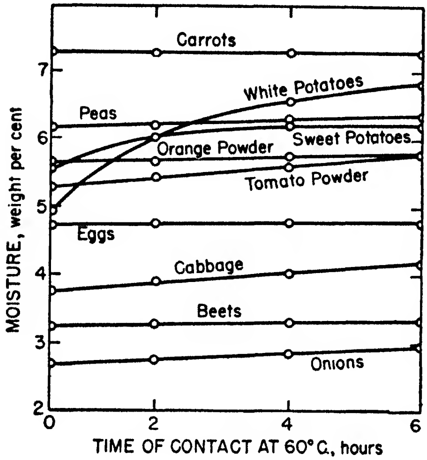


Fig. 34a. Fischer volumetric moisture values of dehydrated foods, using methanol extraction.[42]

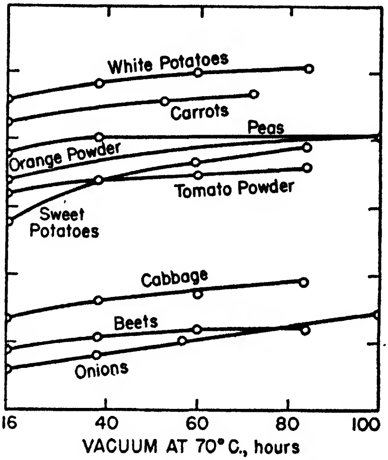


Fig. 34b. Loss in weight of dehydrated foods at 70°C. and less than 100 mm. Hg.[42]

Dried whole egg powder was exceptional; ½ hour soaking in methanol at 22°C. yielded values within 0.1 per cent of those obtained by the vacuum-oven method after ½ hour at 60°C. (Identical results were obtained after 5 hours vacuum-drying at 100°C.) Beets, by the Fischer titration, indicated a slight increase in apparent moisture content, 3.2 per cent water after ½ hour at 60°C. and 3.5 per cent after 24 hours at 60°C. The lower value checked that of the vacuum-oven method after 60 hours, which apparently was the maximum figure obtained. Peas, likewise, yielded a small increase in apparent moisture after prolonged extraction with methanol at 60°C. and, like carrots, gave a moisture value 0.3 to 0.5 per cent higher than that of the maximum oven figure. Orange powder remained at a constant figure (within experimental error) by the volumetric procedure, giving a value checking that of the 38 hour oven weight loss. Onions, cabbages, and tomato powder showed a slow rate of increase in apparent moisture content after prolonged

TABLE LIII

Vacuum-Oven and Volumetric Methods for Determination of Moisture in Dehydrated Vegetables[42]

Material	Apparent moisture, vacuum-oven,[a] wt. %	Moisture found, Fischer titration,[b] wt. %											
		Contact[c] at 22°C., hours							Contact at 60°C., hours				
		0.5	2	16	48	64	88	96	0.5	2	4	6	24
Carrots	6.62	6.33	7.01	7.12	7.14			7.05	7.30	7.31	7.32	7.34	7.29
White potato	6.84	2.81	3.90	5.50	6.16			6.47	4.98	6.09	6.63	6.85	7.26
Cabbage	3.63	3.12	3.15	4.00		4.13	4.20		3.76	3.91	4.00	4.17	4.65
Tomato powder	5.40		4.07	4.98		5.08	5.17		5.32	5.57	5.70	5.85	6.14

[a] Values after 38 hours at 70°C., interpolated from Figure 34.

[b] Samples ground in Wiley mill fitted with a 40-mesh screen.

[c] With methanol.

contact with methanol at 60°C. Sweet potatoes did not attain a limiting value until after 4 hours extraction, while white potatoes proceeded even more slowly.

"Thermal decomposition" in methanol at 60°C. apparently contributed to the slow but constant increase in moisture content such as was found with onions, cabbages, and tomato powder. With white potatoes, however, case-hardening and gelation effects[44] probably reduced considerably the rate at which moisture diffused into the extractant.

In another series of experiments, Johnson[42] added known quantities of water to 1 gram samples of dried foods (ground to 40 mesh) suspended in methanol. The mixtures were maintained at 60°C. for 2 hours and then titrated for water (Table LIV).

TABLE LIV

Titration of Dried Foods to Which Water Had Been Added[42]

Material	Initial water found, mg.[a]	Water added, mg.[b]	Total water, mg.	
			Calculated	Found
Carrots	71.3	84.0	155.3	158.2
	71.3	130.9	202.2	201.0
Orange powder	53.9	80.3	134.2	131.5
	53.9	135.5	189.4	187.8
Onions	29.6	70.4	100.0	98.4
	29.6	117.5	147.1	146.3
Eggs	47.3	76.9	124.2	125.0
	47.3	115.5	162.8	162.0

[a] Average of replicate analyses by Fischer reagent titration.
[b] Control analyses gave recoveries of 99.6 and 100.8 per cent on 86.6 and 133.1 milligrams of water, respectively.

Possible interfering compounds were not present in food materials of these types to account for more than a few hundredths of a per cent of moisture. For example, Johnson demonstrated that the quantity of *l*-ascorbic acid found in these dehydrated foods was equivalent to no more than 0.03 per cent water (see above).

On the basis of these observations, Johnson[42] recommended the following procedure for the determination of moisture in some food materials. The samples, prepared as indicated below and containing 30 to 200 milligrams of water, were weighed into dry glass-stoppered

---

[44] Reeve, R. M., *Food Research*, 8, 128–36 (1943).

250 ml. No. 27 Erlenmeyer flasks. Then 25 ml. of dry methanol were added to each. Depending on the material (see below), the mixtures were either stoppered and allowed to stand at room temperature or connected by means of ground joints to reflux condensers (protected with Drierite). The flasks were heated to 60°C., then removed, quickly stoppered, and stored in an air oven at 59 ± 1°C. for the desired length of time. After the mixtures had cooled to room temperature, Karl Fischer reagent was added to excess, followed by back titration with standard water-in-methanol to the "dead-stop" endpoint.

Substance	Particle size	Extraction conditions
Egg powder	As received	0.5 hr., room temperature
Carrots	4–10 mesh	0.5 hr., 60°C.
Beets	4–10 mesh	0.5 hr., 60°C.
Peas	4–10 mesh	2 hrs., 60°C.
Orange powder	As received	2 hrs., 60° C.
Sweet potato	40 mesh	4–6 hrs., 60°C.
White potato	40 mesh	Unknown
Onion	4–10 mesh	Unknown
Cabbage	4–10 mesh	Unknown
Tomato powder	As received	4–6 hrs., 60°C.

Although the absolute accuracy of Johnson's procedure was uncertain, the method was precise (duplicate determinations consistently checked within a few hundredths of a per cent) and required only a fraction of the time involved in the vacuum-oven method. There was little doubt as to the reliability of the procedure when applied to egg powder, carrots, beets, peas, orange powder, and probably sweet potatoes and tomato powder. White potatoes, cabbages, and onions appeared to reach limiting values after about 24 hours in methanol at 60°C.

Tamm[45] found that dried milk powder, as well as dried egg powder, could be dispersed in methanol and titrated directly with Karl Fischer reagent. His analyses of dehydrated fruits and vegetables were compared with the official A.O.A.C. oven weight loss method,[32] in which the samples were heated for 6 hours at 70°C. and a pressure of 100 mm. or less.

---

[45] Tamm, R. F., Continental Can Co., Chicago, Illinois, *private communication.*

In Tamm's technique,[45] the dehydrated plant material was ground rapidly in a Wiley mill and sieved through a 40-mesh screen. The finely divided product was transferred quickly to a jar, tightly stoppered, and thoroughly mixed. Approximately 2 gram samples, accurately weighed, were transferred to 125 ml. glass-stoppered Erlenmeyer flasks and covered with 25 ml. of dry methanol. The contents of the stoppered flasks were swirled, then allowed to stand at room temperature, with occasional shaking. This extraction period varied with the type of material being analyzed for moisture. Hard and flintlike materials, such as dehydrated sweet potatoes, carrots, hominy, and Irish potatoes, were allowed to stand for 1 hour; 15 to 30 minutes were reported sufficient for the more porous materials, including onions, beets, cabbages, apple nuggets, and cranberries. In general, longer extraction periods should have introduced no errors, provided that the flasks were tightly stoppered. After the minimum required time, the mixture was titrated for moisture with Karl Fischer reagent either visually or electrometrically.

Materials of high water content were analyzed by titration of an aliquot of the supernatant alcohol or by use of a smaller sample.

The effect of particle size on the determination of moisture by the vacuum-oven and Karl Fischer methods is shown in Table LV. Although the apparent moisture in the sweet potatoes as determined by the vacuum-oven method increased regularly with decrease in particle size (probably due in part to thermal decomposition), the Karl Fischer reagent titers remained essentially constant at 20 mesh or smaller.

TABLE LV

Determination of Moisture in Sweet Potatoes[45]

Particle size	Water found, wt. %	
	Vacuum oven, 70°C.[a]	Karl Fischer titration[b]
Ground in Wiley mill, unsieved	4.33	5.68
20 mesh	4.90	5.97
30 mesh	5.33	6.15
40–60 mesh	5.85	5.92
60 mesh	6.85	5.97

[a] Based on 6 hours heating at 100 mm. or less.

[b] After standing for 1 hour with methanol at room temperature.

A few other food materials which were titrated directly with Fischer reagent immediately after being dissolved or dispersed in methanol are reported in Table LVI.

TABLE LVI

Fischer Titration for Moisture in Miscellaneous Food Materials[45]

No.	Substance	Sample size, g.	Water found, wt. %
1	Orange powder	1	2.48 ± 0.01
2	Orange powder		2.74 ± 0.02
3	Orange powder		2.64 ± 0.05
4	Coffee, soluble	0.5	2.08 ± 0.08
5	Coffee, soluble		2.26 ± 0.06
6	Coffee, soluble		3.17 ± 0.05
7	Coffee, soluble		2.61 ± 0.03
8	Coffee, soluble		1.58 ± 0.03
9	Cereal, baby food	0.5	6.36 ± 0.09
10	Cereal, baby food		5.52 ± 0.22
11	Shortening	5	0.42 ± 0.01

The widely different conditions for the extraction of moisture from food materials recommended by Johnson[42] and Tamm[45] are based on comparative oven weight loss data at 38 and 6 hours, respectively. These results, therefore, are not directly comparable but are representative of the present uncertainty in the evaluation of the true moisture content of dehydrated fruits and vegetables.

D. Determination of Moisture in Carbohydrates

Some indication of the behavior of starches was given in the previous section where it was pointed out that vacuum-oven drying techniques—depending somewhat on the conditions used—might not yield true moisture contents (page 182). Sair and Fetzer[37] made a rather extensive study of drying and distillation methods for the analysis of corn starch and its modifications. They concluded that either long (24 hour) toluene distillation or 5 to 20 hour vacuum-oven drying at 100°C. were reliable reference methods and that in some cases rapid oven procedures using temperatures up to 140°C. were satisfactory (*cf.* p. 182). A more thorough investigation of determination of moisture in potato starch was reported by Porter and Willits[43] who included a complete summary of previous investigations by oven and distillation methods. They compared weight loss results from mechanical convection, gravity convection, and vacuum-oven methods with those from distillation methods and the Karl Fischer titration. The potato starch was thoroughly mixed by rolling in a sampling cloth and then stored in moisture-tight Mason jars. For the first analyses, 5 gram portions of the sample were placed in a Brabender moisture tester (a forced draft oven with built-in balance) and heated for periods ranging from 2 to 60 minutes at temperatures ranging from 100° to 170°C. (All analyses were made on portions of this one sample; a constant value of 16.00 per cent moisture was found at 57 and 164 minutes at 100°C.)

Based on postulations of Nelson and Hulett[46] that decompositions of cereals heated at a constant low pressure occur at widely differing rates depending on the temperatures (denoted by breaks in the moisture–temperature curves) but that at lower temperatures the loss from decomposition is slight, a plot was made[43] of loss in weight *versus* temperature using the weight loss values for each temperature (Fig. 35). Using these data as a basis, Porter and Willits assumed that the point of intersection of the two curves of Figure 35 represented the moisture content for the starch sample (16.45 per cent). Comparative data offered by several oven drying methods and by distillation are given in Table LVII. The increased loss in weight (greater than 16.45 per cent) at higher temperatures was postulated as being due to decomposition or deep-seated dehydration of the starch molecule.

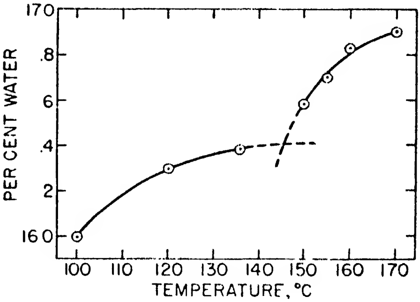


Fig. 35. Loss in weight of potato starch dried at various temperatures in Brabender moisture tester.[43]

In the analyses by the Karl Fischer method, Porter and Willits[43] extracted 1 gram samples of the potato starch for 8 hours at room temperature with 10 ml. of dry methanol in a 125 ml. Erlenmeyer flask covered with rubber dental dam. The titration was carried out by inserting the buret tip through a pinhole in the rubber. The results are recorded in Table LVIII. The average value of 16.06 per cent was 0.37 per cent lower than for the Brabender method but 0.45 per cent higher than that found by gravity convection air oven

---

[46] Nelson, D. A., and Hulett, G. A., *Ind. Eng. Chem., Anal. Ed.,* 12, 40–5 (1940).

TABLE LVII

Summary of Loss in Weight of Potato Starch Dried to Constant Weight at Various Temperatures[43]

Temp., °C.	Type of oven					Distillation[a]
	Brabender	Mechanical convection	Carter-Simon	Gravity convection	Vacuum[c] (5 mm. Hg.)	
76						15.50 (3–5)[b]
80					16.48 (24)[b]	
100	16.00 (1)[b]	16.00 (30)[b]		15.61 (24)[b]	16.57 (5)	
110						16.20 (3–5)
120	16.30 (0.6)					
135	16.40 (0.5)	16.35 (5)	16.24 (0.5)[b]	16.58 (5)		
137						16.33 (3–5)
146						16.40 (3–5)
150	16.55 (0.3)	16.65 (3)		16.66 (0.5)		
155	16.70 (0.3)		16.52 (0.3)			
160	16.85 (0.4)					
170	16.95 (0.3)					
180		16.68 (0.5)				

[a] Distillation with carbon tetrachloride (76°C.), toluene (110–111°C.), xylene (137–140°C.), and tetrachloroethane (146°C.).
[b] Figures in parentheses represent time in hours.
[c] All other ovens operated at 1 atmosphere.

drying at 100°C. (15.61 per cent). Although no vacuum-oven data below 80°C. were reported, it is safe to assume that the weight loss at the 70°C. temperature would likewise be lower (<16.48 per cent). (Refer to data obtained by Johnson[42] on starch food materials, page 187, Chapter VI, and Figure 34 on page 189.)

TABLE LVIII

Moisture in Potato Starch by Fischer Titration[43]

No.	Water found, wt. %
1	16.14
2	16.01
3	16.17
4	16.04
5	16.00
6	16.08
7	16.00
Average	16.06

In the report of this investigation[43] major emphasis was placed on oven drying methods. The results obtained by the single methanol extraction cannot be considered as evidence that low results would always be given by the Fischer titration procedure, even assuming that values given by the method of Figure 35 represent only free water. It would be interesting, for example, to determine the effect of increased temperature for the extraction, following the technique of Johnson.[42] Some indication of the more complete extraction using methanol at 60°C. was included in Johnson's data,[42] in which he compared vacuum-oven drying and Fischer reagent titration on two starches (Table LIX).

Because of the difference in conditions employed by Porter and Willits[43] for the vacuum drying of potato starch, no direct comparison with their Brabender method can be made. (Porter and Willits[43] reported a value of 15.48 per cent after vacuum drying for 1.5 hours at 80°C., and 16.35 per cent after 22 hours. Apparently no intermediate times were noted.) The constancy of results obtained by Johnson, however, suggest complete removal of water which indicates that, for all practical purposes, the methanol extraction of moisture from potato and wheat starch is complete after 30 minutes at 60°C.

TABLE LIX

Determination of Moisture in Starch[42]

Material	Moisture found, wt. %				
	Vacuum-oven method[a]		Fischer titration[b]		
	16 hrs.	100 hrs.	0.5 hr.	6 hrs.	24 hrs.
Potato starch	13.55	13.58	13.41	13.46	13.45
	13.60	13.65	13.43	13.42	13.51
Wheat starch	10.15	10.25	10.21	10.12	10.26
	10.15	10.32	10.08	10.22	10.22

[a] Vacuum-oven values at 70°C. and less than 100 mm. mercury.

[b] Volumetric values after indicated times of contact for approximately 1 gram samples with 25 ml. of methanol at 60°C.

An interesting variation in extraction procedure was suggested by Morell.[1] Up to 10 grams of materials such as yeast, malt syrups, or green malt were added to 200 ml. of dry methanol in a Waring Blendor and mixed at high speed for about 10 minutes. The sample was thus thoroughly ground and simultaneously extracted by the methanol, which practically reached its boiling point due to high-speed stirring. Finally, a convenient aliquot was titrated for water. Morell, because of other duties, was unable to pursue the investigation of the general applicability of this extremely rapid extraction procedure but he obtained sufficiently encouraging results to recommend trying it on those materials from which the water ordinarily was not rapidly extracted by methanol. The technique promises to be widely useful since, with suitable protection against contamination from atmospheric moisture, it should permit rapid analyses of many types of carbohydrates with a high order of precision and accuracy.

The authors[2] developed a modified apparatus ("Hydractor") for the extraction of moisture from porous solids. This unit, shown in Figure 36, consisted of an indented 500 ml. flask and a high-speed stirrer (15,000–18,000 rpm.) mounted through a ₮ 55/50 stainless steel standard taper. In preliminary studies, the sample was weighed into the stoppered ₮ 55/50 flask and 200 ml. of dry methanol were added. The flask was connected to the unit. Dry nitrogen under slight positive pressure was introduced through the tube at the head of the steel taper to maintain a protected atmosphere above the liquid in the flask. The mixture was stirred for 10

TABLE LX

Determination of Moisture in Vegetables[2]

Substance	Moisture found, wt. %	
	Vacuum-oven method[a]	Fischer titration
Cabbages		85.0 ± 0.2
Carrots		79.6 ± 0.2
Celery[b]	5.29	5.99 ± 0.03
Onions[b]	6.25	6.27 ± 0.00
Peas[b]	8.45	8.64 ± 0.02
Sweet potatoes[b]	1.39	3.68 ± 0.01

[a] Six hours at 70°C. and about 5 mm. mercury.
[b] Dehydrated vegetables.

to 15 minutes. Then, either the total contents were titrated in place or a suitable aliquot was removed and titrated, the size of aliquot depending on the total quantity of water present. Typical results on a few of the materials studied initially are given in Table LX.

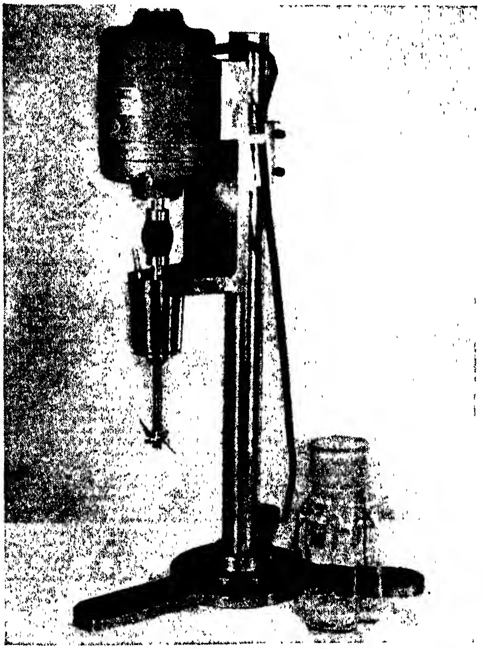


Fig. 36. "Hydractor" for preparing solids for titration.[3]

This unit had several advantages over the Waring Blendor for an extraction of this type. The sample and extractant were always protected and the mixture could be titrated directly in the flask. Furthermore, the stirring could be carried out at a reduced temperature, at which point many normally tacky, viscous materials would be in the form of brittle solids.

A small standard grind side arm on the flask probably would be convenient. This opening would permit liquid transfers with the flask in position and also be more desirable for titration of the total contents of the flask, since the smaller opening would give more protection during titration with Fischer reagent.

Often thermal decomposition tends to take place at elevated temperatures. An extraction apparatus of this type permits rapid reduction of the sample to an extremely fine powder at room temperature or below.

The determination of moisture in sugars has been the subject of a wide variety of proposed oven drying and distillation procedures which were reviewed and studied by Fetzer and his coworkers.[47,48] It is apparent from these and other data[49] that the thermal instability of these materials requires rigidly controlled conditions, which often vary with type of sugar. The Fischer reagent titration, on the other hand, is generally applicable. This was first recognized by Zimmermann[50] who noted that aldoses and

TABLE LXI

Fischer Titration for Moisture in Sugars[50]

No.	Substance	Water, wt. %	
		Found	Calculated
1	Glucose, anhydrous	0.02	
2	Galactose, anhydrous	0.018 ± 0.02	
3	Glucose, monohydrate	9.36 ± 0.03	9.09
4	Maltose, monohydrate	5.66 ± 0.06	5.00
5	Lactose, monohydrate	5.38 ± 0.01	5.00
6	Sorbitol	3.31 ± 0.06	

---

[47] Evans, J. W., and Fetzer, W. R., *Ibid.*, **13**, 855–7 (1941).

[48] Cleland, J. E., and Fetzer, W. R., *Ibid.*, **13**, 858–60 (1941); **14**, 27–30 (1942); **14**, 124–9 (1942).

[49] Smart, S. G., and Mitchell, T. J., *Intern. Sugar J.*, **48**, 68–70 (1945).

[50] Zimmermann, A., *Fette u. Seifen*, **46**, 446–50 (1939).

ketoses did not influence the course of the titration reaction, as indicated in Table LXI.

The purified hydrated sugars were known to contain a fraction of a per cent of mother liquor or surface condensation water in addition to their definite waters of crystallization. The slightly higher values obtained by the titration over those calculated for the hydrate thus represented additional free water. The sorbitol sample was supposedly the hemihydrate. Since only about 75 per cent of the calculated water was found, Zimmermann concluded that there was no definite hydrate of sorbitol stable under atmospheric conditions. Some sugars, such as lactose and maltose, were insoluble in both methanol and the reagent. Zimmermann found that the water contents of these materials could be determined after a preliminary extraction period if the materials were finely ground.

In later publications Almy, Griffin, and Wilcox,[51] Johnson,[42] and Zerban and Sattler[52] reported further investigations on the analysis of sorbitol, sugars and process solutions. (See also the data of Fosnot and Haman[35] on the analysis of malt syrup, page 183, Table XLIX). Almy, Griffin, and Wilcox successfully made determinations on thick syrups and on flexible glue compositions containing polyhydric alcohol plasticizers; previously no satisfactory water method was available. The water contents of the various materials ranged from only a few tenths of a per cent to 70 per cent. Typical results are shown in Table LXII.

The moisture values for the sugars and pectin were based on ½ hour contact with methanol at 60°C. Prolonged contact (up to 24 hours) had no appreciable effect on these values; variations of less than 0.1 per cent were found. The slightly high value for fructose obtained by vacuum-oven drying was probably due to decomposition, as evidenced by darkening of the sample and continued weight loss with prolonged drying, a phenomenon not observed with the other sugars.[42] In comparing water values found by titration with those found by oven drying, Almy and his coworkers[51] observed that the precision of the volumetric method, as indicated by agreement between duplicate and triplicate analyses, was better than that of the oven procedure. Their published data included analyses of 20 sorbitol solutions, including processing liquor, and 7 glues containing

---

[51] Almy, E. G., Griffin, W. C., and Wilcox, C. S., *Ind. Eng. Chem., Anal. Ed.*, 12, 392–6 (1940).

[52] Zerban, F. W., and Sattler, L., *Ibid.*, 18, 138–9 (1946).

polyhydric alcohol plasticizers. Samples of sorbitol syrup, to which known water was added, gave total recoveries in the range of 169.4 to 343 milligrams of water; these values varied from the calculated concentration by a maximum of ±12 milligrams with an average variation of ±4 milligrams.

TABLE LXII

Determination of Water in Sugars and Syrup Solutions

Material	Water found, wt. %		Reference
	Fischer titration	Other methods	
Sucrose	8.31, 8.21	8.37[a]	42
	34.53, 34.93, 34.13	34.59[b]	52
	24.56, 24.65, 24.73	24.21[b]	52
Glucose	3.46, 3.45	3.53[a]	42
Fructose	1.51, 1.52	1.91[a]	42
Pectin	10.00, 10.02	9.86[a], 10.02[c]	42
Dextrose	59.13, 58.92	60.11[b]	52
	59.72, 59.74	59.57[b]	52
Levulose	19.44, 19.83, 19.51	19.92[b]	52
Sorbitol syrup	19.8, 19.8	19.8, 19.9[d]	51
	19.7, 19.5, 19.7	19.9, 20.7, 20.7[d]	51
Commercial invert syrup	29.03, 28.99	28.42[b]	52
Glue, air dried	16.50	16.50, 16.60[e]	51
Glue, composition	30.2, 30.4	30.5[f]	51

[a] Vacuum-oven method, 16 hours at 70°C.
[b] Precision sugar refractometer value.
[c] Vacuum-oven method, 100 hours at 70°C.
[d] Vacuum-oven drying at 65°C.
[e] Oven drying at 110°C.
[f] Calculated from water contents of components.

The glue compositions were insoluble in methanol and in Fischer reagent. Almy[51] found that if these mixtures were first dissolved in glacial acetic acid, a homogeneous solution was maintained throughout the titration. The samples were prepared as follows. About 0.5 to 1 gram of sample was covered with 5 ml. of glacial acetic acid in glass-stoppered extraction flasks and allowed to stand overnight at room temperature. If necessary, the mixture was warmed slightly to effect complete solution and then titrated with Fischer reagent. The excessive heating of the acid mixture was avoided, since under these conditions esterification with the polyhydric alcohol plasticizer led to erroneously high water values. (At room temperature, esterification would be very slow in the absence of a catalyst.)

Crude molasses was titrated visually without interference. Approximately 0.8 gram samples dispersed in 50 ml. of methanol analyzed 22.96 ± 0.01 per cent water in quadruplicate determinations.[2]

Analysis of cotton for moisture by usual oven techniques was time consuming and subject to inaccuracies due to decomposition. A method based on selective hydrolysis of acetic anhydride was reported[53] but this required rigidly controlled, predetermined conditions to prevent acetylation of the cellulose. The ease with which the moisture in cotton could be titrated with Karl Fischer reagent first was reported by Zimmermann.[50] Although no experimental details were given, he recorded values of 6.53 and 6.65 per cent moisture found by titration as opposed to 5.66 and 6.40 per cent weight loss by oven drying after 5 hours at 105°C. and 760 mm. and 10 hours at 105°C. and 5 mm., respectively. Similarly, somewhat higher results for cotton linters by titration (6.09 ± 0.04 per cent) than by oven drying at 102°C. (5.96 per cent) were reported by Mitchell[54] who used the following procedure:

The sample of cotton linters containing 50 to 250 milligrams of water was weighed into a 500 ml. glass-stoppered iodine or Erlenmeyer flask containing 100 ml. of dry methanol. After standing for 30 minutes at room temperature, the mixture was titrated directly with Karl Fischer reagent to a permanent endpoint. After correction for water in the methanol, obtained by titration of 100 ml., the net water found represented the total free water in the sample.

This cold extraction procedure was found to be applicable to the determination of moisture in cotton textiles. Keating and Scott[55] found that a 10 minute contact period of sufficient sample to contain 50 to 250 milligrams of water with 100 ml. of methanol was enough to extract all of the moisture from cotton yarn or fabric. (The 10 minute extraction period was based on experiments with both light- and heavy-weight cotton fabrics for periods from 5 to 90 minutes, with maximum deviations after 10 minutes of 0.09 and 0.10 per cent, respectively.) Comparative data with those of the

---

[53] Mitra, N. C., and Venkataraman, K., *J. Soc. Chem. Ind.*, 57, 306–10 (1938).

[54] Mitchell, J., Jr., *Ind. Eng. Chem., Anal. Ed.*, 12, 390–1 (1940).

[55] Keating, J. F., and Scott, W. M., *Am. Dyestuff Reptr., Proc. Am. Assoc. Textile Chem. Colorists,* 31, No. 13, 308–10 (1942).

standard A.S.T.M. oven drying method using a mechanical convection oven at 105–110°C.[56] are summarized in Table LXIII.

TABLE LXIII

Comparison of Titration Method with Standard Oven-Drying Method for Representative Fabric Samples[55]

Cotton sample	Moisture regain,[a] wt. %		Difference, wt. %
	Oven drying	Fischer titration	
Starch-sized, bleached sheeting	6.97	7.32	0.35
Gray sheeting, 64 × 67	7.70	7.98	0.28
Bleached, unfinished print cloth, 64 × 60	7.56	7.57	0.01
Bleached, mercerized print cloth, 64 × 60	8.91	8.99	0.08
No. 5 oceanic duck	6.99	7.10	0.11
Bleached, unfinished twill, 96 × 64	6.59	6.93	0.34
Bleached, unfinished broadcloth, 100 × 60	6.46	6.82	0.36
"Zelan" treated fabric	7.48	7.56	0.08
Untreated "Zelan" control	8.03	8.40	0.37
40/2 C.P. mercerized yarn	9.17	9.36	0.19
40/2 C.P. unmercerized yarn	7.49	7.76	0.27

[a] Regain: expression for calculation on a dry basis.

In carrying out these analyses no attempt was made to condition the samples in an atmosphere of constant temperature and humidity prior to the determinations. With each fabric, however, the samples for both oven drying and Karl Fischer reagent titration were cut and weighed at the same time under the same atmospheric conditions. The volumetric method appeared to be equally effective whether the cotton was in yarn or fabric form, was gray or bleached, mercerized, finished with starch, or treated with a "Zelan" water-repellent finish. No more than 30 minutes were required for an analysis by the titration method, whereas at least 5 to 7 hours were required to reach constant weight by oven drying. Furthermore, the precision appeared to be better in triplicate determinations. Samples of twill and broadcloth showed an average deviation from the mean titration value of only 0.02 per cent with a maximum deviation of 0.04 per cent; corresponding figures based on oven drying were 0.04 and 0.06 per cent, respectively.[55]

Because of the consistently lower moisture regain values given by the oven drying method as compared with the titration procedure, tests were made to determine whether the titration method would show any residual moisture after oven drying. Three pieces

---

[56] *Am. Soc. Testing Materials, Standards on Textile Materials,* October, 1941, p. 27.

of a cotton broadcloth sample (analyzing 6.34 and 6.14 per cent moisture by Fischer titration and oven drying, respectively) together with 3 control pieces were dried to constant weight; the 3 test pieces were then transferred within the atmosphere of the oven to the standard methanol solutions for the usual 10 minute extraction, followed by titration with Karl Fischer reagent. An average of 0.34 per cent moisture was found by titration of the oven-dried samples.[55] This difference might be a measure of equilibrium moisture in the samples under oven conditions of humidity.

Immediately after the transfer of the first 3 samples to the methanol, the 3 control samples were removed from the oven, cooled in a desiccator, and weighed. The average weight loss figure differed by only 0.06 per cent from the average oven-dried value, indicating that no appreciable quantity of moisture was picked up during the transfer.

TABLE LXIV

Determination of Moisture in Cellulose[54]

Substance	Water, wt. %, Fischer titration	Loss at 102°C., wt. %
α-Cellulose, 98%	5.84 ± 0.07	5.80
α-Cellulose, 95%	4.39 ± 0.06	4.40
Cardboard	4.73 ± 0.08	4.67
Newspaper	5.18 ± 0.06	5.17
Wood pulp, cellophane quality	4.20 ± 0.08	4.18
Wood pulp, rayon type	4.47 ± 0.06	4.44

Similar observations were made on wool.[2] Approximately 1 gram samples of four-ply wool yarn were covered with 100 ml. of dry methanol. After standing for 30 minutes at room temperature, 9.30 ± 0.01 per cent moisture was found by titration, compared to 9.26 ± 0.08 per cent found by oven drying at 102°C.

Regenerated cellulose and paper likewise could be titrated directly with Karl Fischer reagent after a short extraction period. The procedure was essentially the same as that described for cotton linters, except that a 1 hour extraction period at room temperature was necessary[54] (see page 203). The averages of quadruplicate analyses by the methanol extraction–Fischer reagent titration technique at room temperature in all cases compared favorably with the weight loss figures by the A.S.T.M. oven drying method.[57] Typical results are noted in Table LXIV.

---

[57] *Proc. Am. Soc. Testing Materials,* 38, I, 1015 (1938).

A second methanol extraction of rayon-type hardwood pulp and 95 per cent alpha cellulose gave net water values of zero, indicating that all of the water was removed in the first extraction.

Based on data obtained in the authors' laboratory,[2] moisture in impregnated capacitor paper (of the type used for electrical insulation) appears to be completely extracted with methanol in a 1 hour contact period. Comparative data were obtained employing the commonly used 4 hour weight loss method at 102°C. For example, capacitor papers showing weight loss values of 6.77 and 3.42 per cent analyzed 7.48 and 4.26 per cent moisture, respectively, by the volumetric method. The paper with a lower water content was oven dried and then placed in methanol. After the normal 1 hour extraction period, an additional 0.95 per cent moisture was found by titration.[2] (Impregnants in paper, according to McLean and Kohman,[58] apparently prevent complete volatilization of the moisture from within the pores of the paper during oven drying.)

Other investigators have demonstrated the application of the Fischer reagent to the determination of moisture in wood pulp[59] and sawdust.[60] The former analyzed 6.5 and 6.6 per cent by titration, as opposed to 6.3 per cent by oven drying to constant weight at 102°C. In the determination of moisture in sawdust a 10 minute contact period of a 1 gram of sample with 50 ml. of methanol was sufficient to give maximum water values. Although the quantity of water found in sawdust by the volumeteric method (10.4 ± 0.2 per cent) was 0.2 to 0.4 per cent lower than weight loss figures after 18 hours at 100°C., an additional 0.1 per cent water was found on titration of the oven-dried material.[60]

Contact periods varying from 7 to 61 minutes showed no marked differences in the amount of water found by titration (10.4 ± 0.2 per cent). The data showed no marked trend; for example, a 7 minute extraction gave a value of 10.4 per cent; 19 minutes, 10.5 per cent; 21 minutes, 10.2 per cent; 48 minutes, 10.3 per cent, and 61 minutes, 10.6 per cent.

It was postulated that equilibrium distribution of moisture between regenerated cellulose and methanol is established in a relatively short time, due probably to low viscosity and favorable sol-

---

[58] McLean, D. A., and Kohman, G. T., *J. Franklin Inst.*, **229**, 223–34 (1940).

[59] McKinney, C. D., Jr., and Hall, R. T., *Ind. Eng. Chem., Anal. Ed.*, **15**, 460–2 (1943).

[60] Rennie, R. P., and Monkman, J. L., *Can. Chem. Process Inds.*, **1945**, 366–70.

vent or replacement properties of alcohol.[54] This same solvent was used to advantage for the extraction of moisture from native cellulose.[54] For the analysis of natural wood a single sample, cut 2.5 × 1.9 × 0.3 cm. (1 × 0.75 × 0.125 in.) was weighed into a 500 ml. glass-stoppered iodine or Erlenmeyer flask containing 100 ml. of dry methanol. After 2 hours at room temperature, the methanol was decanted into another 500 ml. glass-stoppered flask and the wood was washed with two 10 ml. portions of methanol. An additional 100 ml. of methanol was poured over the wood. This mixture was allowed to stand for 18 hours. The net water found in the two successive methanol extracts was equivalent to the total water removed from the sample. It was desirable to titrate the first extract immediately after decantation. The second extract was not decanted, as the wood was inert toward the reagent.

Results obtained on kiln-dried cypress, air-dried Douglas fir, and partly dried oak are summarized in Table LXV. Total moisture values by Fischer reagent titration usually were somewhat higher than weight loss figures. In order to determine whether additional water could be found in oven-dried wood, duplicate samples of each cut were dried to constant weight at 104°C. and then subjected to a single methanol extraction. Based on the total water present, an

TABLE LXV

Determination of Moisture in Native Wood[54]

Wood	Water by extraction and Fischer titration, wt. %				Loss at 102°C., wt. %[c]
	1st extraction	2nd extraction	3rd extraction	Total	
Cypress[a]	7.68	0.28	0.00	7.96	7.69
	7.63	0.31	0.00	7.94	7.68
Cypress[b]	6.83	0.32	0.00	7.15	6.55
	7.09	0.33	0.00	7.42	6.98
Douglas fir[b]	25.22	0.55	0.00	25.77	24.49
	24.60	0.70	0.00	25.30	24.01
	25.05	0.52	0.00	25.57	24.98
	21.64	0.48	0.00	22.12	22.38
	21.66	0.45	0.00	22.11	21.98
	22.07	0.52	0.00	22.59	
Oak[a]	39.00	0.72	0.00	39.72	39.88
	39.60	0.66	0.00	40.26	40.69
Oak[b]	40.64	0.82	0.00	41.46	41.41
	39.17	0.75	0.00	39.92	40.86

[a] Cut perpendicular to grain (cross cut).
[b] Cut parallel to grain (flat grain).
[c] Dried to constant weight, about 4 hours.

increase in recovery of 5 per cent relative was found in cypress (cross cut), 4 per cent in cypress (flat grain), 2 per cent in Douglas fir (flat grain), and 1 per cent in oak (both cuts). The amount of water remaining in the various woods was surprisingly constant after oven drying at 104°C., even though the type of previous treatment of the wood was different. The results, ranging from 0.3 to 0.5 per cent absolute for the three varieties studied, may have represented equilibrium values under the oven conditions.

The extraction of moisture from wood probably involves two processes: removal of water absorbed on the surface and removal of water from the inner cell walls. The required double extraction seemed to support this theory, further evidence of which was gained from additional experiments.[54]

Individual samples of Douglas fir, approximately 2.5 × 1.9 × 0.6 cm. (1 × 0.75 × 0.25 in.) were cut parallel to the grain and covered with dry methanol. About 97.5 per cent of the total water was obtained in the first extraction for periods ranging from 6 to 80 hours at room temperature (27 ± 3°C.) or 2 to 4 hours of refluxing. An additional 2 per cent was obtained from a second extraction requiring a minimum of 16 hours at room temperature, while the remaining 0.5 per cent required a third treatment of 16 hours. (The longer time required for the second and third extractions may be evidence of more strongly held water.) When the thickness of the wood sample was decreased by one half (to 0.3 cm., 0.125 in.), the third extraction showed no increase in water over that originally present in the methanol.

Using samples of the three types of wood investigated (cypress, Douglas fir, and oak), the percentages of moisture removed in the first extraction were calculated, based on the total amount found by several extractions of a given sample. In Figure 37, moisture removed in the first extraction of the three types of wood is plotted against reciprocal time, as the latter scale is advantageous in showing comparative water removal in the shorter periods of time. In 1 hour about 96 per cent of the total moisture was recovered, while in 3 hours the maximum of 98.5 per cent was attained. While these figures were approximately correct for a considerable range of initial moistures, there was some dependence on initial moisture content. This conclusion is brought out in Figure 37 and is supported by other experiments. For example, the curves for Douglas fir and oak, which had been partially dried to contain 5 to 10 per cent moisture, approached the curve for cypress with 8 per cent initial moisture. The 1.5 to 3.5% of the initial moisture remaining after the first extraction required about 18 hours for complete extraction.

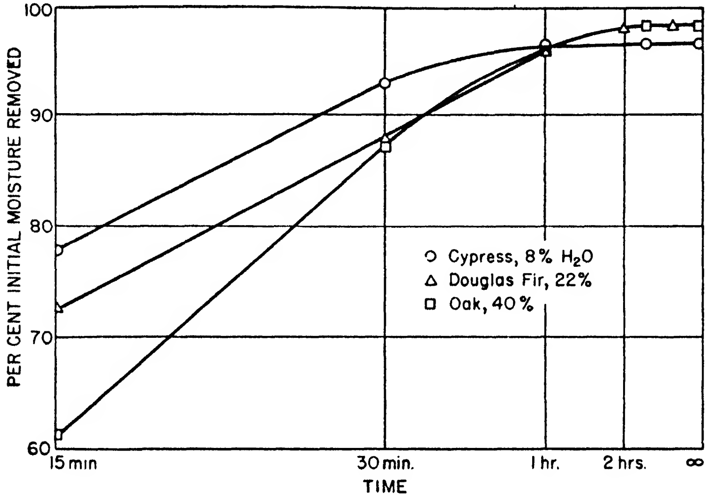


Fig. 37. Removal of initial moisture on first extraction with methanol.[54]

Where approximate results were acceptable, only a single 1 hour cold extraction with methanol was necessary. The values found, when divided by 0.96 (the average percentage of total moisture removed in this time interval), gave a fairly reliable analysis for total moisture in the wood section. Thus, the Fischer reagent technique may be made less time consuming at a small sacrifice in accuracy. However, it does not approach the rapidity of instrumental analyses based on relative conductance or capacity measurements, which give a good approximation of the water content of wood within a few moments. (See Chapter I.)

Usually moisture in leather is determined by oven drying to constant weight at 100°C.[61] or at 165°C.[62] Berkenheim[63] has studied

---

[61] Wallace, E. L., *J. Am. Leather Chem. Assoc.*, **36**, 7–12 (1941).

[62] Gordonova, R. D., *Byull. Glavkozhzamenitel*, No. 4, 33–8 (1939); *C.A.*, **36**, 6039 (1942).

[63] Berkenheim, T. I., *Trudy Moskov. Teknol. Inst. Legkoi Prom. im. L. M. Kaganovicha*, 1941, No. 3, 3–11; *C.A.*, **40**, 3014 (1946).

the applicability of the Karl Fischer reagent titration to the analysis of leather and leather products.*

E. Determination of Moisture in Plastics, Lacquers, Paints, and Varnishes

In general, synthetic resins present some difficulty in the estimation of their water contents. Thermal methods usually introduce depolymerization or degradation reactions giving low-boiling products which are evolved with the moisture. Differential methods based on electrical properties of solids are usually unsatisfactory, for molding powders at least, because of irregular packing effects. Distillation techniques such as that proposed by Phillips and Enas[64] involving entrainers, although time consuming, are usually satisfactory for materials containing more than 0.5 per cent moisture. Cornish[65] found that the Karl Fischer reagent could be used to advantage for the analysis of plastic molding powders. In his method the sample, containing about 60 milligrams of water, was added to a flask containing 20 ml. of "pretitrated" dry methanol. Since the powders were insoluble in the alcohol, the mixture was refluxed for 30 minutes employing a condenser protected with a drying tube. (Either the procedure of Morell[1] using a Waring Blendor or that of the authors employing a 15,000 rpm. stirrer[2] might prove advantageous in extracting moisture from molding powders; see page 198.) At the end of this time the extracted moisture was determined by titration with Karl Fischer reagent. Since the moisture originally present in the methanol was eliminated by preliminary titration, the titer became a direct measure of moisture content of the sample. Powdered plastics analyzed by this technique are given in Table LXVI.

As pointed out earlier, the "pretitration" technique requires a double titration for every sample. Where several samples are to be analyzed, it is better from a time standpoint to use the dry methanol directly, correcting for its small water concentration from separate titration data.

Simultaneous analyses of sample No. 3 by the azeotropic distillation method gave an average value of 1.6 per cent, but the devia-

---

* The authors were unable to locate a copy of Berkenheim's paper in this country; probably the abstract was derived from a foreign source.

[64] Phillips, E., and Enas, J. D., *J. Assoc. Official Agr. Chemists*, **27**, 442–6 (1944).

[65] Cornish, G. R., *Plastics (London)*, **1946**, 99–103.

TABLE LXVI

Fischer Titration for Moisture in Standard Plastic Molding Powders[65]

No.	Material	Sample, g.	Water found, wt. %
1	Urea formaldehyde molding powder	1.000	11.6
		1.000	11.6
		1.000	11.7
2	Phenol formaldehyde molding powder	3.00	4.85
		3.00	4.83
3	High acetyl cellulose acetate, sample A (green)	2.000	1.81
		2.000	1.80
4	High acetyl cellulose acetate, sample B (red)	5.00	1.57
		5.00	1.56
5	High acetyl cellulose acetate, sample C (red)	5.00	1.56
		5.00	1.58
6	Cellulose acetate, dried at 60°C.	5.00	0.24
		5.00	0.25
		5.00	0.26
7	Alvar molding powder (a polyvinyl alcohol resin)	5.00	1.82
		5.00	1.81

tions between consecutive analyses were considerable, results varying by as much as 0.5 per cent absolute. The precision of the Fischer reagent titration method varied from ±3 per cent relative on sample No. 6 to ±0.3 per cent on No. 2. These differences were obviously a function of moisture content and sample size. The figures for dried cellulose acetate, for example, were based on titers of about 1.5 ml. of Fischer reagent. All of these titrations were made using the modified "dead-stop" technique reported by Cornish[65] (pages 93 and 94, Chapter IV). The electrometric method probably was essential for the dyed powders (insofar as the dyes were methanol soluble) but was not necessary for lightly colored materials.

Carter and Williamson[66] demonstrated the applicability of the Fischer volumetric procedure to the estimation of moisture in unidentified synthetic resin solutions. Duplicate analyses on 13 routine samples containing about 1 per cent water checked within ±0.00 to ±0.01 per cent, averaging about ±0.005 per cent.

A technique used in the authors' laboratory which proved to be both rapid and precise for the analysis of many plastic materials was based on dissolving the sample in a selective solvent, such as xylene, before adding the methanol (a nonsolvent). The polymer

---

[66] Carter, R. J., and Williamson, L., *Analyst*, 70, 369–71 (1945).

which precipitated was a finely divided solid from which the moisture was extracted with ease. The heterogeneous mixture was titrated directly with Karl Fischer reagent. Even on a routine basis, duplicate analyses of many polymers and copolymers showed relatively good precision. Various samples of polythene, for example, have analyzed 0.007 ±0.000, 0.125 ±0.015, and 0.0054 ±0.0004 per cent moisture.[2]

McKinney and Hall[59] successfully applied the volumetric method to the analysis of Vinsol resin and rosin size. Samples containing from 50 to 150 milligrams of water were weighed into a 150 ml. distillation flask and 50 ml. of dry methanol were added. The mixture was heated to boiling and then the flask was stoppered during cooling to room temperature, after which the mixture was titrated with Fischer reagent. The Vinsol resin analyzed 1.2 per cent moisture by titration and 1.4 per cent by weight loss, while the rosin size gave values of 30.4 and 30.5 per cent moisture by titration compared with 30.9 per cent by carbon tetrachloride distillation and 29.3 per cent by oven drying.

A tentative A.S.T.M. Karl Fischer technique has been published for the determination of water in lacquer solvents and diluents.[67] In this case a reagent containing one half the standard iodine concentration was suggested for the analysis of samples containing 50 to 80 milligrams of water, equivalent to a titer of 40 to 45 ml. of reagent. The solvent chosen depended on the organic functional groups present in the sample. Ordinarily 10 to 15 ml. of methanol were used. In the presence of carbonyl compounds or amines, however, the methanol was replaced by 25 ml. of pyridine or 10 to 15 ml. of glacial acetic acid, respectively. The use of pyridine to decrease the rate of reaction between the methanol of the reagent and the carbonyl compound usually was satisfactory provided that the titration was rapid (see page 150, Chapter V).

Rennie and Monkman[60] demonstrated the direct applicability of this titrimetric method to shellac–alcohol solutions. Ten analyses of a 4 alcohol : 1 shellac solution analyzed 6.33 per cent moisture with a maximum deviation of 0.02 per cent, while a like number of analyses of a 2 : 1 solution gave values of 5.91 per cent moisture with a maximum deviation of 0.04 per cent. By analyzing shellac solution residues after drying for 24 hours at 50°C. to remove the

---

[67] *Am. Soc. Testing Materials, Standards,* II, 1535 (1944).

water and other volatile constituents, these investigators obtained an average of 0.12 per cent moisture by titration after dilution with dry methanol. Morell[1] likewise reported satisfactory results on shellac and glue–gelatin mixtures.

Swann[68] applied the Fischer reagent titration to the determination of moisture in paints and varnishes. Up to 20 grams of sample were weighed into a 250 ml. glass-stoppered flask. Ten to 25 ml. of dry pyridine (0.1 per cent water or less) were added to both the sample and control flasks, the amount varying according to the viscosity of the sample. Then the mixtures were shaken, 25 to 50 ml. of Karl Fischer reagent ($\approx$ 6 mg. H_2O/ml.) were added, and the stoppered flasks were placed in a bath at 50°C. for 45 minutes, with shaking at 10 minute intervals. After cooling to room temperature, the mixtures were titrated with pyridine containing 0.1 per cent water to the potentiometric endpoint (tungsten–platinum electrodes; see page 83, Chapter IV).

In order to check the accuracy of his method, Swann added known quantities of 1 per cent water in pyridine to samples of an anhydrous paint and an alkyd resin type varnish, heating the mixtures at 50°C. for 45 minutes to insure solution. Portions of the homogeneous mixtures were analyzed by the above method. Comparative data by the standard azeotropic distillation method[69] were also determined on the resin solution. Results are reported in Table LXVII.

TABLE LXVII

Determination of Moisture in Paint and Varnish[68]

Substance	Titration sample, g.	Water, wt. %		
		Present	Found	
			Fischer titration	Distillation[a]
Paint	6.9320	0.21	0.22	
	8.014	0.24	0.24	
	12.4703	0.51	0.51	
Alkyd resin type varnish	7.4141	1.65	1.65	1.45
	4.8950	0.82	0.82	0.70
	6.0911	0.44	0.44	0.31

[a] Sample weights varied from 89.8 to 114.4 grams.

---

[68] Swan, M. H., *Ind. Eng. Chem., Anal. Ed.*, 18, 799–800 (1946).
[69] Federal Specification TT-P-141a, Method 408 (June 16, 1944).

Swann used his method for the analysis of many types of paint, enamels, and resins. Resins, which in isolated form were insoluble in pyridine, first were dissolved in a mutual solvent. Zinc oxide was the only powdered pigment found which interfered in the titration (see Chapter VIII for reactions of oxides).

F. Determination of Moisture in Naval Stores

The Fischer reagent is generally applicable to the analysis of Naval Stores. None of the functional groups normally encountered, such as ethylenic unsaturation, hydroxyl, and carboxyl groups, offer any interference.

Distillation methods have been proposed for the analysis of these materials[70] but often the quantity of water involved was too small to permit accurate analyses by these procedures. This was rather conclusively demonstrated in the results reported by Grotlisch and Burstein[71] and are shown in part in Table LXVIII.

TABLE LXVIII

Moisture Content of Pine Oils and Terpene Solvents[71]

No.	Sample	Moisture found, wt. %	
		Distillation[a]	Fischer titration
1	Steam-distilled amber pine oil	0.45	0.46, 0.46
3	Synthetic pine oil, A	1.08	0.87, 0.87
4	Synthetic pine oil, B	0.51	0.50, 0.52
5	Destructively distilled pine oil	0.44	0.42, 0.41
7	Commercial terpineol	0.43	0.42, 0.44
8	Fenchyl alcohol		0.78, 0.80
9	Redistilled pine oil	0.00	0.13
10	Redistilled terpineol	0.00	0.075
11	Gum spirits of turpentine, fresh		0.012, 0.015
15	Gum spirits of turpentine, very old		0.380[b]
16	Steam-distilled wood turpentine		0.022, 0.026
22	Pinane		0.031
24	α-Pinene		0.081
25	β-Pinene		0.13
26	Dipentene, old		0.25

[a] 100 ml. of sample used; all results were for single determinations.

[b] Decomposed reagent at room temperature; test satisfactory on cooling to 5°C.

A limited number of materials of this type have been included in other papers. For example, pine oil gave values of 0.66 and 0.64 per cent water by titration, compared with 0.5 per cent by distillation.[59]

---

[70] *Am. Soc. Testing Materials, Standards,* III, 230 (1939).

[71] Grotlisch, V. E., and Burstein, H. N., *Ind. Eng. Chem., Anal. Ed.,* 17, 382–3 (1945).

Grotlisch and Burstein's reverse technique[71] for the analysis of pine oil did not appear to offer any advantage over the usual procedure. In their method a known volume (10 ml.) of Fischer reagent was added to a glass-stoppered Erlenmeyer flask and titrated with the sample of pine oil until the red-brown iodine color just disappeared. The weight of added pine oil was determined from its density or by weighing the stoppered flask before and after the addition of sample.

The extremely sensitive Fischer reagent was more likely to absorb atmospheric moisture by this technique since the reagent was not well protected during the analysis. Pine oil was quite soluble in methanol, so that it probably would have been better to dissolve the oil in a known volume of the dry alcohol and titrate directly with Fischer reagent.

When pinene and related hydrocarbons were titrated directly (without added diluent), the Karl Fischer reagent endproducts were precipitated, obscuring the endpoint. By dissolving these materials in pyridine, Grotlisch and Burstein[71] were able to maintain a homogeneous solution throughout the titration. Turpentine was soluble in pyridine but, since the former and the methanol of the reagent were immiscible, a two-phase liquid system appeared during the titration. The lower layer, containing methanol with some pyridine and the inorganic compounds, extracted the moisture from the terpene. At the endpoint, the lower layer assumed the red-brown color of excess iodine while the upper turpentine layer remained nearly colorless.

Grotlisch and Burstein "pretitrated" pyridine in a glass-stoppered flask, weighed, added the turpentine, and then weighed the mixture. Under these conditions a sample of highly oxidized turpentine (probably containing peroxide) reacted with the hydriodic acid of spent Fischer reagent, liberating free iodine. This interference was minimized by slowly adding the sample to the pyridine cooled in an ice bath (Table LXVIII).

If weighing is considered necessary, it would probably be simpler and safer to eliminate the "pretitration" step so that the turpentine would not be in contact with the hydriodic acid before titration. Then one of two courses could be followed. The two-phase system could be titrated directly (at reduced temperature if necessary) or, after a suitable extraction period with agitation, the methanol layer could be separated and titrated, correcting for the moisture content of the methanol by a blank titration. The time requirement for extraction of the moisture could be derived from experiments involving a second extraction with the final conditions based on those in which the second extract showed no increase in water over that already present in the methanol.

The accuracy of this technique was determined by check analyses of gum turpentine, which was dried by refluxing over calcium oxide and redistilled, to which known quantities of water were added. Samples calculated to contain 0.020, 0.034, 0.062, and 0.089 per cent water analyzed 0.020, 0.034, 0.062, and 0.090 per cent, respectively.

In those cases where the pyridine acts as a common solvent its use is desirable. With turpentine, however, it probably would be just as satisfactory to employ methanol alone as extraction medium, titrating as above to the endpoint in the alcohol layer. In both cases the mixture should be agitated vigorously before and during the titration. A permanent endpoint is sufficient evidence that all of the moisture has been extracted from the terpene layer.

G. Determination of Moisture in Explosives

Low-temperature azeotropic distillation, desiccation, oven drying, and the Karl Fischer reagent titration appear to be the only techniques used for the determination of moisture in explosives and related compounds. McKinney and Hall[59] demonstrated the use of the volumetric method on both nitroglycerin and nitrocellulose. The former analyzed 0.28 and 0.29 per cent water (0.29 per cent by desiccation) and the latter, 2.8 per cent in duplicate (2.9 per cent by distillation). Johnson and Hutchison[72] made a more thorough investigation of the applicability of the Fischer reagent titration to the determination of moisture in nitroglycerin. Typical results, based on direct titration of 8 to 27 gram samples, compared to the standard desiccation method were:

Water by desiccation, wt. %	Water by Fischer titration, wt. %
0.08	0.08, 0.08
0.18	0.16, 0.15
0.19	0.20, 0.20
0.29	0.28, 0.28

A thorough investigation of the Karl Fischer method for the analysis of grained gunpowder was made by Rennie and Monkman.[60] Time experiments in which 1.5 gram samples were covered with 10 ml. portions of dry methanol and allowed to stand at room tempera-

---

[72] Johnson, C. L., and Hutchison, G. F., Eastern Laboratory, E. I. du Pont de Nemours & Co., Inc., *private communication.*

ture demonstrated that about 35 minutes were required for complete extraction of the moisture. (Variable results were found at contact times up to 30 minutes; the maximum value was found after 34 minutes, remaining constant for periods over an hour in length.) Six individual samples were titrated after this time interval, giving results of 0.945 ± 0.005 per cent water, while weight loss figures (2 hours at 100°C.) averaged 1.03%. This significant difference between methods may have represented loss of other volatile constituents during the drying period, particularly when it was shown that gunpowder, dried for 18 hours at 100°C., gave positive values by the Fischer titration (one value of 0.18 and two of 0.020 per cent).

In applying the volumetric method to the determination of moisture in priming mixtures, containing potassium chlorate and mercury fulminate, Bradford, Stauffer, and Summers[73] found that a direct suspension technique could not be used, because of the interfering reactions of the strong oxidizing or reducing agents present. They adopted an extraction procedure in which the sample was permitted to settle and a portion of the supernatant clear liquid was removed for titration. Some of the components were soluble in methanol and consequently this alcohol could not be used for the extraction. Three other alcohols and dioxane were tried, using the following extraction procedure:

The sample, containing from 100 to 200 milligrams of water, was weighed to the nearest milligram into a glass-stoppered high-form weighing bottle. Then 50 ml. of dry solvent were added, the bottle was stoppered, and the contents were swirled cautiously for a few minutes until the solid was dispersed thoroughly.

The agitation of the sample constituted the only hazardous part of the analysis. Great care was taken to prevent particles of the primer from being shaken up between the ground-glass surfaces of the bottle and stopper. A gasket-type seal would probably tend to minimize this potential hazard.

After the solid had settled (up to 2 hours) 25 ml. of the clear supernatant liquid were withdrawn carefully and titrated with Fischer reagent. Typical results are given in Table LXIX, showing recovery of known water added to samples of an anhydrous priming mixture. Ethanol appeared to be the most satisfactory from the standpoints of efficiency in extraction and ease of titration. Several priming mix-

---

[73] Bradford, C. I., Stauffer, W. O., and Summers, L., Remington Arms Co., Bridgeport, Conn., *unpublished results.*

TABLE LXIX

Fischer Titration for Moisture in Priming Mixture[73]

Dry sample, g.	Solvent	Water, g.		Relative error, %
		Added	Found	
3.682	Ethanol	0.211	0.210	−0.5
2.594	Ethanol	0.213	0.212	−0.5
3.667	Ethanol	0.213	0.213	0.0
4.069	*n*-Propanol	0.214	0.212	−1.0
2.815	*n*-Propanol	0.207	0.204	−1.5
3.675	*n*-Propanol	0.210	0.206	−2.0
3.272	*tert*-Butanol	0.213	0.210	−1.5
2.928	*tert*-Butanol	0.207	0.205	−1.0
3.646	*tert*-Butanol	0.215	0.215	0.0
3.110	Dioxane	0.202	0.200	−1.0
3.760	Dioxane	0.206	0.202	−2.0
3.459	Dioxane	0.206	0.202	−2.0

TABLE LXX

Determination of Moisture in Explosive Compounds[73]

Substance	Water added, wt. %	Water found, wt. %	
		Drying method	Fischer titration
Priming explosive No. 1	0.0	11.9 ± 0.1[a,b]	12.4 ± 0.1[b]
Priming explosive No. 1, previously dried over calcium chloride	0.0		0.12
	0.95[c]		0.85
	0.80[c]		0.70
	0.70[c]		0.60
Priming explosive No. 2	0.0	8.8 ± 0.1[a,b]	8.9 ± 0.2[b]
PETN,[e] wet	0.0	39.5 ± 0.6[b,d]	41.8 ± 0.7[b]
Tetrazene, wet	0.0	38.6 ± 0.1[a,b]	40.2 ± 0.9[b]
Lead styphnate	0.0		25.2 ± 0.2[b]

[a] Desiccation over calcium chloride at room temperature.
[b] Average of three or four individual determinations.
[c] Includes initial water found.
[d] Vacuum drying at 110°C.
[e] Pentaerythritol tetranitrate.

tures and other explosive components were analyzed by the ethanol extraction technique and by selected drying methods. Typical results are given in Table LXX.

Another sample of priming explosive, extracted with dioxane, analyzed 11.3 ± 0.1 per cent moisture, compared to 11.0 ± 0.1 per cent by desiccation over calcium chloride. Methanol was satisfactory for the extraction of wet tetrazene, giving a relative precision of 0.2 per cent in triplicate determinations.

For the estimation of moisture in "Nitramon" primers, Featherer[74] weighed approximately 10 gram samples into stoppered Erlenmeyer flasks. Then 100 ml. of methanol were added and the mixture was titrated directly with Fischer reagent. Typical results compared to azeotropic distillation with carbon tetrachloride are given below. Included also are data obtained on samples which had been dried for 6 hours at 70°C. and then allowed to absorb various amounts of water:

Water added, wt. %	Water by Fischer titration, wt. %	Water by distillation with CCl_4, wt. %
0.00	0.02	0.06, 0.07
0.00	0.00	0.06, 0.07
0.37	0.30	
0.34	0.29	
0.18	0.15	
0.07	0.06	
0.09	0.07	
0.94	0.94	
1.01	1.01	

In general the Fischer reagent titration results appeared to be slightly high.

A few experiments were made on smokeless powder. Five gram samples were weighed into 250 ml. iodine flasks and 100 ml. of dry methanol were added. After standing at room temperature for periods ranging from about 10 minutes to 24 hours, 50 ml. portions of the alcohol were removed and titrated with Fischer reagent. Results were constant after extraction for 1 hour. A comparison of values by the titration method, allowing about 1 hour for extraction, with those of weight loss after 2 hours at 100°C. showed the latter to be between 0.13 to 0.16 per cent absolute higher in the range of 0.5 per cent water. This difference, at least in part, was due to loss of other volatile matter.[55]

---

[74] Featherer, R. L., Eastern Laboratory, E. I. du Pont de Nemours & Co., Inc., *private communication.*

Hardy, Bonner, and Noyes[75] compared four different procedures for the estimation of moisture in solvent-free, double-base powders (that is, mixtures of nitrocellulose and nitroglycerin to which small percentages of stabilizers, plasticizers, and inorganic salts had been added). In the first method, 10 gram samples 1 to 2 mm. thick were placed in a desiccator over concentrated sulfuric acid and allowed to come to constant weight. At least 4 days were required for an analysis, although the actual analyst's time was less than that required for any of the other procedures studied. In the second procedure, 10 gram samples of like thickness were placed in a shallow aluminum dish with a close fitting glass cone. The system was placed on a closed, steam-heated bath at atmospheric pressure. After 2 hours, the unit was removed and cooled overnight in a desiccator.[76] The third technique involved azeotropic distillation with carbon tetrachloride of a 100 gram sample. The fourth was based on a Fischer reagent titration: five gram samples approximately 1 mm. thick were added to an electrometric titration flask (see page 79, Chapter IV) containing 60 ml. of "pretitrated" 1 : 1 ether–methanol or 1 : 1 pyridine–ethyl acetate. The mixture was stirred for 30 to 60 minutes, a measured excess of Karl Fischer reagent was added, and after 1 minute of stirring the mixture was back titrated electrometrically with standard water-in-methanol. Results by three of these procedures, given in Table LXXI, show comparative values on a sample of unknown moisture content. Additional data on samples previously desiccated to constant weight and rehumidified are included. Hardy and his coworkers[75] concluded that the desiccation and Karl Fischer procedures were the most reliable.

Nitrated ethyleneurea has been analyzed successfully by the Fischer titration method.[2] About 2 gram samples or less, depending on the moisture content, were weighed into glass-stoppered flasks, covered with 50 ml. of dry methanol, and after standing for about 10 minutes with occasional swirling were titrated with Fischer reagent. Duplicate samples, analyzing 0.15 ± 0.01 per cent water initially, gave values of 14.5 ± 0.2 per cent, after a calculated 14.5 per cent water had been added, including original moisture found.

---

[75] Hardy, J., Bonner, W. D., Jr., and Noyes, R. M., *Ind. Eng. Chem.*, **18**, 751–3 (1946).

[76] Picatinny Arsenal, Tentative Specification PXS-633 (Rev. 5), December 16, 1941.

TABLE LXXI

Estimation of Moisture in Smokeless Powder[75]

Sample description	Gain in weight on rehumidification, %	Moisture, wt. %		
		Desiccation method	Cone-and-dish method	Karl Fischer titration[a]
Run No. 1		0.275		0.29
2		0.265		0.29
3		0.260		0.27
4		0.260		0.28
5		0.285		0.29
6		0.285		0.29, 0.29
Average		0.270		0.29
Desiccated			0.080	0.020
Rehumidified	0.205	0.220	0.235	0.235
Rehumidified	0.325	0.320	0.315	0.340
Rehumidified	0.500	0.495	0.480	0.495
Redesiccated				0.010

[a] Runs 1 through 5, using ether–methanol; run 6, using pyridine–ethyl acetate.

Attempts to use the visual endpoint for the determination of moisture in trinitrotoluene were unsuccessful.[72] Samples dispersed in methanol developed a deep red color during titration with Fischer reagent, which obscured the endpoint.

This color development did not necessarily indicate an interfering reaction. It would be interesting to try one of the electrometric procedures on TNT.

H. Miscellaneous Applications

Another application of the titrimetric procedure was for the determination of moisture in soap. Ivory flakes (3 grams) which were dispersed in 50 ml. of methanol and titrated immediately with Karl Fischer reagent analyzed 3.02 ± 0.02 per cent moisture in duplicate determinations, compared to 2.99 per cent by azeotropic distillation with xylene.[2] In materials of this type, any free caustic would interfere, appearing as 1 mole of apparent water per mole of caustic present. Where the percentage of free base was known, suitable corrections could be applied to the Karl Fischer reagent titer (see Chapter VIII).

Preliminary data on the determination of moisture in powdered coal were inconclusive.[2] Two samples, analyzing about 4 per cent moisture by weight loss at 105°C. after 1 hour in a gravity convection oven, gave values of about 6 per cent by titration. In the latter analysis, 1 gram samples were dispersed in 30 ml. of dry methanol, excess Karl Fischer reagent was added immediately, and the mixture was back titrated electrometrically with standard water-in-methanol.

CHAPTER VII

Determination of Water in Inorganic Compounds

The use of Karl Fischer reagent for the quantitative determination of water in organic compounds has been discussed in the previous chapters. In the inorganic field, the reagent affords a valuable means of determining the water present in hydrated salts, absorbed on the surfaces of solids, or contained in solutions with inorganic substances. Preliminary data were given by Karl Fisher in his original paper;[1] more extended information was presented by the authors and coworkers.[2] There were a number of inorganic compounds which reacted with Fischer reagent to indicate fictitious water contents, but these compounds were readily classified (Chapter VIII) and in most cases were subject to exact correction.

Where applicable, the Fischer titration method was more rapid and direct than the commonly used thermal drying procedures. It was also free from the difficulty of finding a drying temperature which permitted complete removal of moisture without the danger of losing other volatile constituents. The Fischer reagent at room temperature had the ability to react completely with water, which was evidently tightly held by inorganic compounds; this could be explained, at least in part, by the tendency of methanol to replace water in such compounds. Thus, the removal of water from hydrated salts and similar materials was greatly facilitated by the large excess of methanol normally present in the reagent and usually employed as solvent or dispersing agent. Even where large coordination affinities or free energies of adsorption for water existed, methanol apparently was able to reduce appreciably the forces opposing the removal of water. For comparison, it has already been demonstrated (page 207, Chapter VI) that methanol extraction removed more water from native and processed cellulose than could be removed

---

[1] Fischer, Karl, *Angew. Chem.*, 48, 394–6 (1935).

[2] Bryant, W. M. D., Mitchell, J., Jr., Smith, D. M., and Ashby, E. C., *J. Am. Chem. Soc.*, 63, 2924–7 (1941).

conveniently by thermal drying under standard conditions at 102°C. The distribution established between moisture in the solvent and in the pores of the solid was sufficiently favorable that it was not essential for the cellulose sample to be present during the subsequent Fischer titration of the methanol extract. On the other hand, even with excess Karl Fischer reagent present, only 96.5 per cent removal was obtained on the first extraction under the otherwise corresponding conditions.

The types of inorganic compounds which may be titrated directly with Fischer reagent for water of hydration or absorbed moisture are summarized in Table LXXII.

TABLE LXXII

Inorganic Compounds Applicable to Fischer Reagent Titration for Water[2,3]

Class	Examples
Salts of organic acids	$Na(CH_3)SO_4$, CH_3COONa, $K_2C_2O_4$, $UL_2(OOCCH_3)_2$, $Na_2C_4H_4O_6$, $NaOOC(CH_2)_4COONa$
Salts of inorganic acids	$NH_4H_2PO_4$, $CdCl_2$, Na_2SO_4, KF, NH_4NO_3, $Na_2S_2O_4$, KSCN, $FeSO_4$, $CaHPO_4$, NaI, $Na_2S_2O_6$, $KMnO_4$, $CaCO_3$, $CaCl_2$, FeF_3
Desiccants	
Acid oxides	SiO_2, Al_2O_3
Mixtures	Clay, Fuller's earth
Inorganic acids and anhydrides	SO_2, HI, HCl, HF, HNO_3, HCN, H_2SO_4, HSO_3NH_2.

Analytical Procedure for Solids. A sample of the powdered compound equivalent to 5 to 10 millimoles (90 to 180 milligrams) of water is dissolved or suspended in 25 ml. of dry methanol (<0.1 per cent water) in a 250 ml. glass-stoppered volumetric flask and the mixture is titrated with standard Karl Fischer reagent to the usual light brown endpoint. The water content of the methanol solvent is determined by a separate titration of an equal volume and the titer of the sample is diminished by the proper amount. The corrected titer is equivalent to the available water in the sample.

A. Determination of Water in Salts of Organic Acids

These compounds in general were titrated readily with the Fischer reagent. They were usually soluble in both methanol and the re-

---

[3] Unpublished results from the authors' laboratory.

agent, and could be titrated directly to a permanent endpoint. Results obtained on representative, nearly neutral compounds are given in Table LXXIII.

Some of these salts were dehydrated at relatively low temperatures and, consequently, the samples frequently were mixtures of the hydrate with anhydrous material. This was particularly noticeable with sodium nitrobenzoate for which two crystalline species were observed microsopically. Comparison of the data for the volumetric method with those for the weight loss procedure indicates rather clearly that in most cases studied the free water plus water of hydration were determined specifically by direct titration with Karl Fischer reagent.

Free or adsorbed moisture on metal salts of nonhydrated organic compounds in general could be titrated precisely and accurately. An interesting industrial application was the determination of moisture in penicillin sodium salt by Levy, Murtaugh, and Rosenblatt.[4] The penicillin salt was thermally unstable and hygroscopic, containing from 0.5 to 1.5 per cent water when properly prepared.

An acceptable method for the analysis of the valuable penicillin required the use of a minimum of sample while maintaining excellent precision and accuracy. The only other feasible method for moisture determination was based on vacuum desiccation over phosphorus pentoxide which required a drying period of 6 to 9 days and included at least 4 weighings on a microbalance.[4] In addition to being time consuming and tedious, the desiccation method did not give precise results readily due to the pronounced hygroscopicity of the salt. The rapid titrimetric method of Levy and his coworkers[4] required only about 200 milligrams of sample (the contents of one ampul); 1 to 25 milligrams of water were titrated with a precision of 20 to 100 micrograms. The container itself, a 25 ml. bottle capped with a rubber stopper, was used as the titration flask, eliminating the necessity for sample transfer. In this microelectrometric technique (page 99, Chapter IV), the hypodermic needle tips of the microburet containing Fischer reagent and the pressure equalizing vent, respectively, were inserted through the rubber cap of the sample bottle. After the addition of a slight excess of Fischer reagent, the needles were withdrawn and the contents were shaken

---

[4] Levy, G. B., Murtaugh, J. J., and Rosenblatt, M., *Ind. Eng. Chem., Anal. Ed.,* 17, 193–5 (1945).

TABLE LXXIII

Determination of Water of Hydration in Salts of Organic Acids

No.	Hydrate	Moles of water found/mole salt		Thermal stability from literature[b]
		Fischer titration	Loss at 110°C.	
1	Barium acetate $Ba(OOCCH_3)_2 \cdot H_2O$	0.27 ± 0.00[a]	0.24 ± 0.00	anh. 41°C.
2	Cadmium acetate $Cd(OOCCH_3)_2 \cdot 2H_2O$	1.99 ± 0.01		
3	Cobalt acetate $Co(OOCCH_3)_2 \cdot 4H_2O$	3.98 ± 0.02		
4	Lead acetate $Pb(OOCCH_3)_2 \cdot 3H_2O$	3.01 ± 0.01[a]		anh. 75°C.
5	Magnesium acetate $Mg(OOCCH_3)_2 \cdot 4H_2O$	4.08 ± 0.01		
6	Nickel acetate $Ni(OOCCH_3)_2 \cdot 4H_2O$	3.97 ± 0.01		
7	Sodium acetate $NaOOCCH_3 \cdot 3H_2O$	2.99 ± 0.00[a]	3.00 ± 0.01	
8	Zinc acetate $Zn(OOCCH_3)_2 \cdot 2H_2O$	2.00 ± 0.00		
9	Uranyl acetate $UO_2(OOCCH_3)_2 \cdot 2H_2O$	2.00 ± 0.02[a]		anh. 110°C.
10	Sodium citrate $Na_3C_6H_5O_7 \cdot 2H_2O$	1.98 ± 0.02		

11	Calcium lactate $Ca(C_3H_5O_3)_2 \cdot 5H_2O$	4.97 ± 0.02		$-3\ H_2O$, 100°C.
12	Calcium malonate $CH_2(COO)_2Ca \cdot 4H_2O$	2.38 ± 0.03	2.14 ± 0.12	
13	Sodium naphthionate $1\text{-}NH_2C_{10}H_6\text{-}4\text{-}SO_3Na \cdot 0.5H_2O$	0.50 ± 0.03	0.32	
14	Sodium *m*-nitrobenzoate $NO_2 \cdot C_6H_4 \cdot COONa \cdot 3H_2O$	1.12 ± 0.01	1.08 ± 0.08	
15	Ammonium oxalate $(NH_4)_2C_2O_4 \cdot H_2O$	1.01 ± 0.00[a]		
16	Potassium oxalate $K_2C_2O_4 \cdot H_2O$	1.03 ± 0.02		
17	Calcium propionate $Ca(OOCCH_2CH_3)_2 \cdot H_2O$	1.00 ± 0.01		
18	Sodium succinate $(CH_2COONa)_2 \cdot 6H_2O$	5.65 ± 0.02	5.65 ± 0.05	
19	Sodium monosulfo-*m*-benzoate $HOOC \cdot C_6H_4 \cdot SO_3Na \cdot 3H_2O$	0.59 ± 0.01	1.56 ± 0.16,[c] 0.55[d]	
20	Sodium formaldehyde sulfoxylate $CH_2OHOSONa \cdot 2H_2O$	2.05 ± 0.60		

---

[a] From the literature[2]; other compounds not previously reported[3].

[b] Data from N. A. Lange, *Handbook of Chemistry*, 4th ed., Handbook Publishers, Inc., Sandusky, Ohio, 1941.

[c] Apparent decomposition on oven drying; strong sulfurlike odor.

[d] By azeotropic distillation with toluene.

TABLE LXXIV

Determination of Moisture in Penicillin Sodium Salt[4]

Lot No.	Sample weight, mg.	Water found	
		Mg.	Wt. %
	Titration with Karl Fischer reagent		
J43	466	6.08	1.30
	466	6.21	1.33
	466	6.21	1.33
	466	6.31	1.35
			Average 1.32_8 *S.D.* 0.021
66	227	3.57	1.57
	227	3.53	1.56
	227	3.57	1.57
	227	3.60	1.59
			Average 1.57_3 *S.D.* 0.013
126	484	2.52	0.52
	484	2.56	0.53
	484	2.52	0.52
	484	2.52	0.52
			Average 0.52_3 *S.D.* 0.005
	Vacuum drying over phosphorus pentoxide		
66	227	4.02	1.77
	227	3.64	1.60
	227	3.91	1.72
	227	3.95	1.74
			Average 1.70_8 *S.D.* 0.075

until the sample had dissolved. Then two 23-gage hypodermic needles were inserted through the stopper and a 7.5 cm. (approximate) length of 30-gage platinum wire was passed through each needle. The needles were withdrawn leaving the platinum wires in contact with the homogeneous solution. After connecting the wires to the electrometric indicator system, the pressure equalizing needle and the needle of the buret containing standard water-in-methanol were inserted. (Fig. 38 shows a detailed photograph of the assembly.) The excess Fischer reagent was determined by back titration to the "dead-stop" endpoint.

Analytical data on three lots of penicillin sodium salt are given in Table LXXIV. Four bottles from each lot were analyzed and for one lot (No. 66) analyses also were made by the phosphorus pentoxide desiccation method. The mean value of the moisture content obtained by the vacuum-drying procedure was significantly greater than the mean obtained by the Fischer reagent titration method. The higher value by vacuum drying was probably due to absorption of moisture from the atmosphere during the transfer step. (This source of error was eliminated entirely in the volumetric method, since no transfer was involved.) It is evident that the technique employing Karl Fischer reagent resulted in a great increase in precision and a considerable saving in time and labor.

Fig. 38. Penicillin bottle used as titrating vessel.[4]

Examples of the other types of nonhydrated salts which have been titrated successfully for adsorbed moisture are given below:[3]

Salt	Water found, wt. %	Salt	Water found, wt. %
1. Ammonium acetate	0.86 ± 0.03	6. Sodium formate	0.05 ± 0.00
2. Sodium adipate	0.07 ± 0.00	7. Potassium acid phthalate	0.06 ± 0.00
3. Potassium bitartrate	0.11 ± 0.01		
4. Ammonium citrate	0.14 ± 0.00	8. Zinc stearate	0.29 ± 0.00
5. Sodium cyclohexane carboxylate	0.06 ± 0.00	9. Sodium methyl sulfate	0.50 ± 0.01

Another interesting application of the Fischer reagent was in the estimation of water and sodium hydroxide in sodium methylate.[5]

Water absorbed by solid sodium methylate reacted to form sodium hydroxide. In methanol solution, however, equilibrium could be established:

$$NaOCH_3 + H_2O \rightleftharpoons NaOH + CH_3OH \qquad (1)$$

As explained in Chapter VIII, sodium hydroxide reacted mole for mole with the iodine of Fischer reagent and, consequently, the titration of a methanol solution represented the total water plus sodium hydroxide, which would be equivalent to total sodium hydroxide in the anhydrous solid sample, allowing for the water introduced with the methanol. (The determination of water plus sodium hydroxide might be a critical factor in the evaluation of sodium methylate solution as a catalyst and as an acidimetric reagent, such as for the titration of acid anhydrides[6] and carboxylic acids in the presence of easily hydrolyzed esters.[7])

The direct titration of relatively pure sodium methylate, dissolved in methanol alone, was characterized by a limited fading of the endpoint which tended to give slightly high results. This difficulty was eliminated by treating the methanol solution of the sample with excess glacial acetic acid or spent Karl Fischer reagent. With acetic acid the bases were neutralized according to the reactions:

$$NaOCH_3 + CH_3COOH \longrightarrow NaOOCCH_3 + CH_3OH \qquad (2)$$

$$NaOH + CH_3COOH \longrightarrow NaOOCCH_3 + H_2O \qquad (3)$$

The reactions of the spent reagent were analogous to that of the acetic acid. When the sample, dissolved in methanol alone, was titrated with Fischer reagent the interfering reaction proceeded until a small amount of spent reagent was present to combine with the alkoxide.

Sodium carbonate, formed through reaction of CO_2 from the air with any caustic present, was apparently insoluble in methanol. If left in the sample, however, this salt would react quantitatively with acetic acid or Fischer reagent, appearing as water:

$$2\,NaOH + CO_2 \longrightarrow Na_2CO_3 + H_2O \qquad (4)$$

$$Na_2CO_3 + 2\,CH_3COOH \longrightarrow 2\,NaOOCCH_3 + H_2O + CO_2 \qquad (4a)$$

Since the reaction of sodium methylate and acetic acid or spent reagent did not involve water, the net Fischer reagent titer (in the

---

[5] Smith, D. M., Bryant, W. M. D., and Mitchell, J., Jr., paper presented before the Analytical section, 100th meeting of the American Chemical Society, Detroit, Mich., September 10, 1940.

[6] Smith, D. M., and Bryant, W. M. D., *J. Am. Chem. Soc.*, **58**, **2453–4** (1936).

[7] Schultz, R. F., *Ibid.*, **61**, **1443–7** (1939).

absence of carbonate) was equivalent to the total water plus caustic in the methanol solution or the total caustic in the dry powdered sodium methylate. In the presence of carbonate, the net titer would be a measure of total caustic plus carbonate.

B. Determination of Water in Inorganic Salts

A severe test of the titration method was made by analyzing hydrated salts of inorganic acids, particularly those which were dehydrated only at elevated temperatures. These compounds were often insoluble or only partially soluble in the dispersing agent. The ease with which hydrate water was titrated by Karl Fischer reagent depended more upon the solubility of the initial or final material in the reaction environment than upon the stability of the hydrate. (In general, compounds containing 6 or more molecules of water of hydration were soluble in methanol and remained in solution throughout the titration.) Many hydrates of high stability were attacked rapidly by the reagent. Also, relatively insoluble hydrates often were dehydrated completely provided that the samples were finely ground and the mixtures were shaken mechanically, preferably with excess reagent.

As long as the sample was soluble, the first endpoint on titration with Fischer reagent was permanent and corresponded to the total water present. Those cases involving a heterogeneous titration mixture, however, usually required either a stepwise titration or mechanical shaking with excess reagent at room temperature, and final back titration with a standard solution of water-in-methanol. For direct titrations, endpoints stable for 15 minutes usually indicated complete reactions. When the initial titration was incomplete, as indicated by a fading endpoint, the mixture was titrated at 10 to 15 minute intervals until a permanent brown color was reached. The choice of liquid dispersing agent assumed some importance for insoluble substances. Glycol, for example, exerted a greater solvent action than methanol on some salts.

Analytical results for a wide variety of inorganic salts are given in Table LXXV. Inspection of this table shows the close agreement either between the Fischer titration results and calculated water content or the Fischer titration results and weight loss on oven drying at 110°C. for those cases in which the latter method was known to succeed. A severe test of the titration method has been

TABLE LXXV

Analytical Data for Inorganic Hydrates

No.	Hydrate	Moles of water found/mole salt		Thermal stability from literature[c]
		Fischer titration	Loss at 110°C.	
1	Sodium bisulfate[a] $NaHSO_4 \cdot H_2O$	1.08 ± 0.01		
2	Barium chloride[a] $BaCl_2 \cdot 2H_2O$	1.98 ± 0.02	1.96 ± 0.01	anh. 100°C.
3	Cadmium chloride[a] $CdCl_2 \cdot 2\frac{1}{2}H_2O$	2.55 ± 0.00	2.50 (132°C.)	anh. 120–130°C.
4	Cobalt chloride $CoCl_2 \cdot 6H_2O$	5.90 ± 0.03	5.91	anh. 110°C.
5	Stannic chloride[a] $SnCl_4 \cdot 5H_2O$	4.97 ± 0.02		
6	Strontium chloride[a] $SrCl_2 \cdot 6H_2O$	5.86 ± 0.02	5.87 ± 0.01 (132°C.)	
7	Sodium dithionate[a] $Na_2S_2O_6 \cdot 2H_2O$	1.69 ± 0.01	1.69 ± 0.01	anh. 110°C.[d]
8	Potassium ferrocyanide[a] $K_4Fe(CN)_6 \cdot 3H_2O$	3.11 ± 0.00	3.14	anh. 70°C.
9	Boron trifluoride $BF_3 \cdot 2H_2O$	2.00 ± 0.01		
10	Potassium fluoride[a,b] $KF \cdot 2H_2O$	1.98 ± 0.03		
11	Chromic nitrate $Cr(NO_3)_3 \cdot 9H_2O$	9.06 ± 0.00		dec. 100°C.
12	Cobalt nitrate[a] $Co(NO_3)_2 \cdot 6H_2O$	6.04 ± 0.02		dec. −3 H_2O, 55°C.
13	Mercurous nitrate[e] $HgNO_3 \cdot H_2O$	0.73 ± 0.00		dec. 100°C.
14	Sodium nitroprusside $Na_2Fe(CN)_5NO \cdot 2H_2O$	1.97 ± 0.01		
15	Calcium diphosphate $CaHPO_4 \cdot 2H_2O$	2.07 ± 0.02		dec.

16	Calcium phosphate[a,b] $CaH_4(PO_4)_2 \cdot H_2O$	0.98 ± 0.01	0.98	anh. 100°C.
17	Manganese ammonium phosphate $MnNH_4PO_4 \cdot H_2O$	0.90 ± 0.01		
18	Sodium phosphate[a] $NaH_2PO_4 \cdot H_2O$	1.01 ± 0.00		anh. 100°C.
19	Sodium phosphate[a] $Na_2HPO_4 \cdot 12H_2O$	11.04 ± 0.03	11.07 ± 0.05	anh. 100°C.
20	Silicotungstic acid $SiO_2 \cdot 12WO_3 \cdot 26H_2O$	24.4 ± 0.10		
21	Aluminum sulfate $Al_2(SO_4)_3 \cdot 18H_2O$	18.15 ± 0.10		dec. 86.5°C.
22	Calcium sulfate[a] $CaSO_4 \cdot 2H_2O$	1.47 ± 0.04	1.06 ± 0.06	anh. 163°C.
23	Cobalt sulfate[a] $CoSO_4 \cdot 7H_2O$	6.99 ± 0.01		anh. 420°C.
24	Ferrous sulfate[a] $FeSO_4 \cdot 7H_2O$	7.00 ± 0.02		anh. 300°C.
25	Ferrous ammonium sulfate[a] $FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6H_2O$	6.01 ± 0.01		
26	Magnesium sulfate[a] $MgSO_4 \cdot 7H_2O$	7.01 ± 0.02	5.00 ± 0.02	anh. 280°C.
27	Manganese sulfate[a] $MnSO_4 \cdot 4H_2O$	4.03 ± 0.02		anh. 280°C.
28	Nickel sulfate[a] $NiSO_4 \cdot 6H_2O$	6.11 ± 0.03		anh. 280°C.
29	Zinc sulfate[a] $ZnSO_4 \cdot 7H_2O$	6.94 ± 0.02	5.70	anh. 280°C.

---

[a] From the literature[2]; other compounds not previously reported[3].

[b] Slow reaction, probably due to insolubility in methanol.

[c] Data from N. A. Lange, *Handbook of Chemistry*, 4th ed., Handbook Publishers, Inc., Sandusky, Ohio, 1941.

[d] Data from C. D. Hodgman, *Handbook of Chemistry and Physics*, 27th ed., Chemical Rubber Publishing Company, Cleveland, Ohio, 1943.

[e] Salt as received yellow in color, indicative of some decomposition.

found in the group of hydrated sulfates of the metals extending from manganese to zinc in the periodic table. These hydrated salts retained the last mole of water up to temperatures approaching 300°C., but titration with Fischer reagent was rapid and complete. Sulfates of this group which were titrated successfully but which required an additional correction for their oxidizing effect are indicated in Chapter VIII.

Another group of hydrates, illustrated in Table LXXV, was readily analyzed by the volumetric technique but not by thermal drying; this included salts undergoing chemical changes such as hydrolysis or pyrolysis on heating. Stannic chloride, cobaltous nitrate, and mercurous nitrate belong to this group.

The hydrates of potassium fluoride, barium chloride, monocalcium phosphate, and calcium sulfate were slow in their reactions with Karl Fischer reagent. Increased rates were observed with the first two hydrates after they were suspended in ethylene glycol instead of methanol.

The incomplete recovery of water from calcium sulfate was of some interest. The data reported in Table LXXV were based on treatment of the dihydrate for 1 hour with excess reagent. Further treatment for 4 hours, however, reduced the residual water content of the salt to 0.36 mole. Caspari[8] effectively demonstrated that the supposed lower hydrates of calcium sulfate, for example, $2\ CaSO_4{\cdot}H_2O$, were merely zeolitic adsorption complexes involving water and a trigonal modification of anhydrous calcium sulfate, more reactive than the better known orthorhombic mineral, anhydrite. Caspari's calculations suggested that the entry or departure of zeolitic water from the trigonal calcium sulfate lattice would be slow because of the small interatomic clearances. The sluggish action of Fischer reagent might have been due to the increased difficulty of replacing water by the more bulky methanol molecule. The Fischer titration gave no real evidence of hemihydrate formation on partial dehydration of $CaSO_4{\cdot}2H_2O$.[8]

Free water was determined in the presence of calcium phosphate[9] (3.9 per cent by titration *versus* 3.6 per cent by oven drying), calcium carbonate[1] (0.23 per cent *versus* 0.24 per cent), and ammoni-

---

[8] Caspari, W. A., *Proc. Roy. Soc. London,* **A155**, 41–8 (1936).

[9] Fosnot, R. H., and Haman, R. W., *Cereal Chemistry,* **22**, 41–9 (1945).

um nitrate[10] (0.26 per cent *versus* 0.245 per cent). In the authors' laboratory[3] it has been found that moisture can be determined in the presence of mono- and diammonium phosphates, ammonium sulfate, ammonium hexanitrato cerate [$(NH_4)_2Ce(NO_3)_6$], potassium ferricyanide [$K_3Fe(CN)_6$], potassium thiocyanate, potassium permanganate, sodium sulfate, sodium hyposulfate ($Na_2S_2O_4$), potassium pyrosulfate ($K_2S_2O_7$), and ferric fluoride (FeF_3).

C. Determination of Water in Desiccants

The successful use of Fischer reagent with salts suggested the possibility of assaying desiccants of the anhydrous salt or absorbent gel type. Fischer[1] demonstrated that Fuller's earth could be titrated, and the authors and coworkers[2] showed that other materials could be assayed (Table LXXVI). In the latter case, the powdered products after exposure to moist air were sampled, suspended in methanol, and titrated without mechanical shaking. The success of the titration method with the first four substances was quite clear, while the less satisfactory behavior with Drierite was consistent with the data for pure calcium sulfate in Table LXXV.

TABLE LXXVI

Analytical Data for Desiccants

Material	Water found, wt. %			Reference
	Fischer titration	Thermal drying at 104°C., 4 hrs.	Thermal drying at 150°C., 4 hrs.	
Fuller's earth	15.6		15.5 (120°)	1
Fuller's earth treated with water	22.4		22.6 (120°)	1
Fuller's earth, dried	1.6		0.0 (120°)	1
Activated alumina	7.02 ± 0.00[a]	5.37 ± 0.06	6.12 ± 0.01	2
Silica gel	5.48 ± 0.01[a]	4.32 ± 0.08	4.71 ± 0.19	2
Calcium chloride	11.28 ± 0.08[a]	2.08 ± 0.06	10.75 ± 0.09	2
Drierite	5.31 ± 0.05[a,b]	5.50 ± 0.10	6.42 ± 0.12	2

[a] Average deviation for duplicate analyses.

[b] Slow reaction characteristic of calcium sulfate, 4 hours.

D. Determination of Water in Inorganic Acidic Materials

Although not many inorganic acidic compounds have been investigated, at least two—sulfur dioxide and hydrogen chloride—have

---

[10] McKinney, C. D., Jr., and Hall, R. T., *Ind. Eng. Chem., Anal. Ed.*, 15, 460–2 (1943).

TABLE LXXVII

Determination of Moisture in Liquid Sulfur Dioxide[1]

Sample	Water found, vol. %		
	Fischer titration	Calcium hydride method	Calculated
Liquid sulfur dioxide	0.012	0.012	
Benzene–sulfur dioxide (75:25)	0.031	0.030	0.033
Benzene–sulfur dioxide (50:50)	0.023	0.020	0.027
Benzene–sulfur dioxide (25:75)	0.017	0.015	0.020
Benzene (50 ml.) + sulfur dioxide (50 ml.) + methanol (10 ml.)	0.115	Not possible[a]	0.118

[a] Interference from methanol.

been studied in great detail. Fischer[1] gave figures on the estimation of moisture in liquid sulfur dioxide (Table LXXVII). No experimental details were given by Fischer but the results showed excellent accuracy. Other procedures proposed for the determination of moisture in sulfur dioxide included evaporation methods in which the water vapor was absorbed by phosphorus pentoxide[11–13] and a thermal technique based on measurement of the temperature at which hydrate crystals emerged from the liquid sulfur dioxide to form on the surface.[14] All of these methods were subject to error according to Zavarov[15] who proposed a Fischer titration procedure and a combined phosphorus pentoxide–Fischer titration method. In his direct titration method, Zavarov collected the sample in a 1 liter Dewar flask fitted with a three-hole rubber stopper; one opening contained a 12 mm. glass tube extending from the bottom of the flask to about halfway through the stopper, allowing room for a glass rod plug; a second opening contained a short length of glass tubing used for filling the Dewar, protected with a stoppered desiccant tube, while the third opening was fitted with a short bent tube. This last tube led to an enlargement filled with phosphorus pentoxide distributed on asbestos and then to a rubber aspirator bulb.

---

[11] Flenner, A., and Keverly, V., *Refrigerating Engineering,* 1931, 5.
[12] Eustas, F. A., *Ind. Eng. Chem., Anal. Ed.,* 5, 77–9 (1933).
[13] Scribner, A., *Ibid.,* 3, 255–7 (1931).
[14] Woog, P., Sigwalt, R., and de Saint-Mars, J., *Bull. soc. chim.,* 53, 1522–4 (1933).
[15] Zavarov, G. V., *Zavodskaya Lab.,* 5, 1314–18 (1936).

The titration vessel, shown in Figure 39, consisted of a vacuum jacketed reservoir, E; a ground glass tube, D, containing phosphorus pentoxide; and a three-way stopcock, C. In carrying out an analysis the dried titration vessel was weighed. (A wooden support contained a center opening in which tube A was placed; this allowed the vessel to be held in an upright position on the balance.) Then the glass plug was removed from the stopper of the Dewar flask containing the sample and tube A was inserted (snug fit) in its place. Stopcock C was turned to the A-B position and, on applying pressure by means of the aspirator bulb, liquid sulfur dioxide was forced up the tube and expelled through tube B (to a waste cold trap) until all moisture on the walls of the tubes had been washed away. On turning stopcock C to connect A and E only, a small portion of the sample was forced into the reservoir, after which the flask was shaken and the liquid was discharged through B. Then about 50 grams of sample were collected in reservoir E, and the vessel was removed, placed in the stand, and reweighed. Finally the protecting tube D was removed, the Fischer reagent buret tip was inserted, and the sample was titrated to the first appearance of unused iodine. This titer was a direct measure of the moisture content of the sample. About 25 minutes was required for the complete analysis, including sampling.

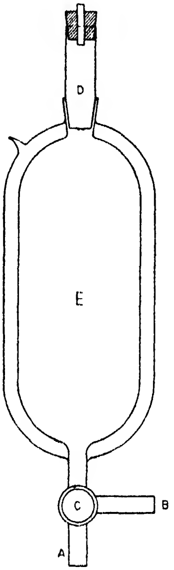


Fig. 39. Vessel for titration of liquid sulfur dioxide with Karl Fischer reagent.[15]

The accuracy of the method was checked by adding known quantities of water in glycerol solution to the sample in the Dewar flask, followed by the standard procedure for the introduction of sample into the titration vessel. Typical results are shown in Table LXXVIII.

Zavarov[15] found that samples containing more than 0.01 per cent moisture on evaporation always left a residue which contained an appreciable quantity of water. The volatile moisture was determined by absorption on phosphorus pentoxide. The residual moisture was

TABLE LXXVIII

Fischer Titration Data for Sulfur Dioxide[15]

Moisture found in initial sulfur dioxide, wt. %	Total weight of sulfur dioxide, g.	Glycerol (containing 6.4% water) added, g.	Water, wt. %	
			Calculated[c]	Found
0.016[a]	664.5	1.3174	0.029	0.030
0.014[b]	502.0	1.5168	0.034	0.034

[a] Average of 4 determinations.
[b] Average of 3 determinations.
[c] Water added plus water found initially.

determined by titration with Karl Fischer reagent. Comparative data are given in Table LXXIX.

TABLE LXXIX

Determination of Moisture in Liquid Sulfur Dioxide[15]

No.	Moisture found, wt. %		
	Volatile moisture only (evaporation of 500 g. over phosphorus pentoxide)[a]	Volatile plus residual moisture (combined method)	Direct Fischer titration of 50 g.
1	0.021	0.036	0.036
2	0.011	0.014	0.014
3	0.010	0.018	0.019
4	0.009	0.017	0.017

[a] Standard evaporation method.

Results obtained by the standard evaporation method[11] were variable but consistently lower than those obtained by the combined and direct titration methods. Zavarov[15] noted that the direct Fischer titration method tended to give high results, since a certain amount of Fischer reagent was required to impart the iodine color. This was observed to be about 0.3 ml. of 0.1 *M* iodine which, for a 50 gram sample, corresponded to 0.001 per cent moisture. He, therefore, recommended the combined method for those cases where higher accuracy was required, particularly with samples of extremely low moisture content. The latter technique used a 500 gram sample, sufficiently large for the iodine correction factor to be negligible. Zavarov implied a high order of precision. Although no figures were given, duplicate titers of the indicated magnitude should have checked within 0.1 ml. The sensitivity of the more rapid direct titration method could obviously be improved by the use of larger samples in the presence of dry methanol as an inert solvent. Separate titration of the methanol control for its water content would tend to cancel a large portion of the constant 0.3 ml. error noted by Zavarov. This could also be reduced by the use of a back titration procedure, in which excess Fischer reagent was added, followed by back titration with standard water-in-methanol.

Aqueous solutions of hydrochloric acid could be weighed directly into methanol and titrated. For example, a 0.3 gram sample of 37.3 per cent hydrochloric acid dissolved in 25 ml. of dry methanol analyzed 62.61 ± 0.02 per cent water on direct titration with Fischer reagent.[3]

In the absence of an inhibitor the sample should be titrated immediately after dilution, since on standing the acid and alcohol might react to form methyl chloride and water:

$$CH_3OH + HCl \longrightarrow CH_3Cl + H_2O \qquad (5)$$

The rate of reaction would tend to increase with increasing acid concentration. Addition of a few milliliters of pyridine is sufficient to inhibit this reaction.

In this case acidimetric techniques based on hydrolysis of acyl halides or acid anhydrides are impractical for accurate moisture determinations because of the extremely large corrections for the acidity of the samples.

Hydrogen chloride gas required a more elaborate technique. Milberger, Uhrig, Becker, and Levin[16] demonstrated that the gas could be absorbed effectively in a pyridine–methanol solvent, the pyridine serving as an acid acceptor and the methanol acting as a solvent, maintaining a homogeneous solution throughout the analysis. Excess pyridine was found to be essential for, in the presence of appreciable quantities of uncombined hydrogen chloride, a deep red color was produced on titration with Fischer reagent. This color could not be discharged even after addition of a large excess of water. (This indicated a color-forming side reaction; a considerable amount of heat was evolved on addition of Fischer reagent.) In this procedure,[16] a 75 ml. portion of 53 volume per cent pyridine-in-methanol was transferred to a 300 ml. ₸ 24/40 Erlenmeyer flask and made anhydrous by the addition of excess Fischer reagent followed

---

[16] Milberger, E. C., Uhrig, K., Becker, H. C., and Levin, H., paper presented before the Division of Analytical and Microchemistry, 110th meeting of the American Chemical Society, Chicago, Ill., September 10, 1946.

by back titration to the yellow endpoint with water-in-methanol solution. (These authors used a visual back titration technique; the direct titration would be equally satisfactory.) Then, a known volume of Fischer reagent was added in sufficient volume to be in excess of that required to react with all of the water in the sample (30 ml. of reagent was sufficient for a 10 gram sample containing 0.4 per cent water). The flask (Fig. 40) was fitted with the glass delivery tube, the ends of which were stoppered to exclude moisture; the unit weighed to the nearest 0.05 gram. The flask was then connected to the hydrogen chloride line. With the stopcock closed and the bleeder line open, the valves from the hydrogen chloride line were opened. Then the stopcock was opened and the rate of flow, controlled by the screw clamp on the bleeder line, was adjusted so that the resulting fumes did not rise more than 2 in. above the liquid level in the flask. (A small positive head of pressure prevented the solvent from rising into the delivery tube as the gas dissolved.) At this rate of flow, a 10 to 15 gram sample was collected in as many minutes. After sufficient sample had been collected, the valve was closed and the delivery tube and flask assembly were quickly removed to prevent liquid from backing up. The flask and the delivery

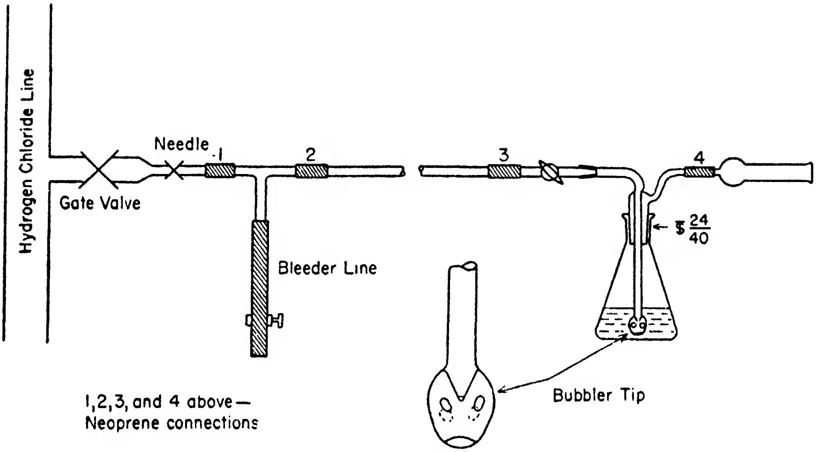


Fig. 40. Apparatus for the determination of moisture in hydrogen chloride gas.[14]

tube, with the ends again stoppered, were reweighed to determine (by difference) the amount of sample collected.

After removal of the delivery tube, the flask was stoppered and shaken until the gas fumes were dissolved. The excess Fischer reagent was determined by back titration with standard water-in-methanol. (A closed system was employed by placing a two-hole rubber stopper in the flask through which a mechanical stirrer and buret tip were inserted.) Both the Fischer reagent standardization and the volumetric relationship of water-in-methanol to reagent were determined in the same apparatus. The net Fischer reagent used up corresponded to the moisture present in the sample. In this procedure no correction for water in the solvent was necessary since the solvent solution was "pretitrated."

When a back titration technique is used, it is essential that excess Fischer reagent be present after addition of the sample. For those who prefer the direct titration method the sample may be added to slight excess (i.e., until the iodine color is just discharged) and then, after weighing, Fischer reagent is added until the iodine color just reappears. In this case the total reagent consumed is a measure of the moisture concentration of the sample.

Results obtained on several samples of commercial anhydrous hydrogen chloride are presented in Table LXXX.

TABLE LXXX

Water in Hydrogen Chloride from Commercial Cylinders[16,a]

Weight of sample, g.	Fischer reagent, ml.	Water-in-methanol soln., back titer	Fischer reagent consumed[b]	Water found	
				Mg.	Wt. %
14.3	5.0	2.23	0.1	0.1	0.001
18.2	5.0	2.5	−0.5	−0.5	−0.003[c]
16.4	5.0	2.15	0.25	0.3	0.002
15.3	5.0	2.17	0.22	0.2	0.002

[a] Ratio of Fischer reagent to water-in-methanol solution = 2.20 ml. per ml.

[b] Reagent equivalent to 1.07 mg. water per ml.

[c] Based on the sample size chosen, the uncertainty in these results must be ≥ 0.003 per cent.

Samples containing known moisture were prepared by passing the gas through a "humidifier" containing a known amount of water which was volatilized into the gas stream with the aid of a microburner. Typical results are given in Table LXXXI.

TABLE LXXXI

Analysis of Hydrogen Chloride Containing Known Moisture[16a]

Sample wt., g.	Water, mg.[a]		Water, wt. %[a]	
	Added	Found	Added	Found
10.7	38.8	39.4	0.37	0.38
11.5	84.4	83.8	0.73	0.73
12.5	73.9	74.6	0.59	0.60
8.8	22.0	22.8	0.25	0.26
12.4	26.5	27.0	0.21	0.22
12.1	53.9	54.7	0.45	0.45
13.5	1.9	1.4	0.01	0.01
16.2	6.3	6.8	0.04	0.04
12.2[b]	37.5	38.6	0.31	0.32
15.0[b]	60.7	61.4	0.40	0.41
13.0[b]	64.6	65.9	0.50	0.51
14.4[b]	25.4	26.6	0.18	0.18

[a] Based on known water added, uncorrected for initial moisture content of the gas.

[b] Contained about 10 per cent of C_4 hydrocarbon gases.

Samples of gas from petroleum refinery operations, for which this method was developed, should contain only hydrogen chloride and moisture, with not more than small amounts of hydrocarbon gases. The last four analyses reported in Table LXXXI contained C_4 hydrocarbons (*n*-butane, isobutane, isobutene, 2-butene, and butadiene). In order to prevent loss through volatilization of hydrocarbons from the sample, the flask was cooled to about —15 to —20°C. by means of an ice–salt bath during the addition of the sample. At this temperature, the solvent prevented the escape of the hydrocarbons. If lower boiling hydrocarbons were present, the exhaust gases could be metered, for inclusion by calculation in the sample weight.

The average difference between found and calculated water contents of the samples reported in Table LXXXI, exclusive of the two containing less than 10 milligrams of water, was 2.1 per cent, with a maximum error of 4.7 per cent. (If necessary, accuracy could have been improved simply by increasing the sample size.) The time required to conduct an analysis was 30 to 40 minutes.

These investigators demonstrated that the presence of sulfur compounds such as hydrogen sulfide and methyl or ethyl mercaptan introduced errors in the analysis. As pointed out in Chapter V, the interfering reactions were quantitative, so that unless present in great excess suitable corrections could be applied.

The authors (page 151, Chapter V) have already shown that moisture could be determined in hydrocyanic acid by direct titration in pyridine or dioxane solution. (These reagents were used to permit the determination of water in carbonyl compounds.) Yoe and Lindsley[17] analyzed a sample of hydrogen cyanide containing moisture, 0.06 per cent phosphoric acid, and 0.43 per cent sulfur dioxide (as H_2SO_4). Then they found that the Fischer titration of the sample dissolved in methanol was faster and more precise than the usual evaporation method where the water vapor was absorbed on phosphorus pentoxide or Drierite. In order to evaluate the volumetric method, Yoe and Lindsley[17] transferred two 5 ml. and two 2 ml. samples of liquid hydrogen cyanide to four 100 ml. glass-stoppered flasks containing 25 ml. of dry methanol. The solutions were titrated directly with Fischer reagent to the visual endpoint. Net titers, after correction for water in the methanol, were equivalent to 2.56 ± 0.01 per cent water. Simultaneous analyses based on absorption of moisture by phosphorus pentoxide were 2.64 ± 0.04 per cent in 2 determinations and those based on weight loss over Drierite were 2.69 ± 0.16 per cent in 7 determinations. These authors concluded that all three methods were accurate to ±0.1 per cent. The Fischer procedure was considered more rapid, precise, and probably more accurate than the gravimetric methods.

Aqueous hydrofluoric acid could be titrated directly.[3] Approximately 0.3 gram samples of 48.3 ± 0.01 per cent hydrofluoric acid, dissolved in 25 ml. of dry methanol, analyzed 51.5, 51.5, and 52.0 per cent water. Moisture in dry hydrogen fluoride (B.P. 19.5°C.) also has been determined by titration with Karl Fischer reagent.[18] The sample was collected in a 3 × 7/8 in. aluminum bomb fitted with a Hofer valve and a special brass tee, one arm of which was threaded to connect with the valve and the other two arms, to connect with $^3/_{16}$ inch copper tubing. (The total weight of the bomb, valve, and tee was approximately 175 grams.) The bomb containing the sample was weighed to the nearest milligram and connected to the apparatus as shown in Figure 41. With the bomb valve closed, anhydrous nitrogen was allowed to flow through the system at a

---

[17] Yoe, J. H., and Lindsley, C. H., Office of Publication Board, Rept. PB 5957 (1944); *Science and Ind.*, 1, 348 (1946).

[18] *Hydrofluoric Acid Alkylation.* Phillips Petroleum Company, Bartlesville, Oklahoma, 1946.

rate of about 3 bubbles per second. Without interrupting the flow of nitrogen the three-way stopcock was turned to permit the addition of 6.0 ml. of dry C.P. pyridine into the 50 ml. test tube.

The test tube was fitted with a two-hole cork stopper to accommodate the 3/16 in. silver delivery tube and the Fischer reagent buret tip; the latter hole formed a loose fit with the buret tip.

Karl Fischer reagent was added to the first appearance of unused iodine and the volume was recorded. Then an additional 10 ml. of Fischer reagent was drawn into the test tube and the bomb valve was opened carefully to permit the sample to be carried by the nitrogen into the test tube until the color of the contents changed from dark brown to light yellow. At this point the valve was closed and the tee warmed with a small alcohol lamp or gas burner to drive out the residual acid. This in turn was washed down into the test tube by the further addition of 2.0 ml. of pyridine. After sufficient time had been allowed for drainage, the contents of the test tube were titrated again with Fischer reagent to the visual endpoint. The bomb was removed and weighed, after the tee

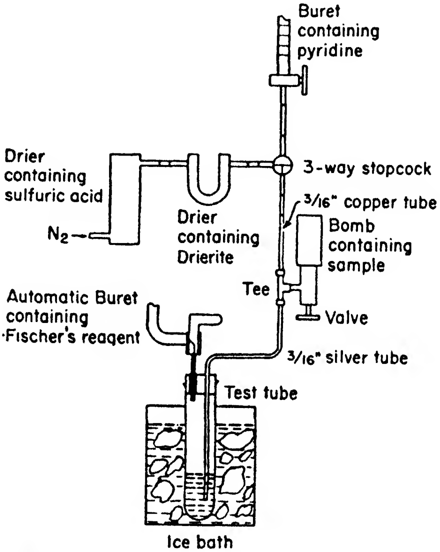


Fig. 41. Apparatus for the determination of moisture in hydrogen fluoride gas.[18]

was cleaned with a pipe cleaner. Sample weight was determined by difference.

The weight per cent moisture in the hydrogen fluoride was calculated from the relation:

$$\frac{(T - 1.33\,P)\,F}{S} \times 100$$

where T = total ml. of Karl Fischer reagent used, P = ml. of Fischer reagent required for 6.0 ml. of pyridine, F = concentration of Fischer reagent calculated as grams of water per ml., and S = weight of sample in grams.

The volumetric method also has been applied to the determination of water in several other acids.[3] Sulfamic acid (HSO_3NH_2) could be titrated directly. For nitric acid, a 0.4 gram sample was treated in the cold (ice bath) with 10 ml. of pyridine and titrated directly with Fischer reagent. An average of 31.25 per cent water was found in a sample analyzing 68.74 per cent nitric acid by standard alkali titration.

Concentrated sulfuric acid was also preferably titrated in the form of the pyridine salt. Several 4 gram samples of 95.5 wt. % acid were cooled in an ice bath and 30 ml. of 1 : 1 pyridine: dioxane were carefully added to each. (Dioxane was added to aid in dispersing the pyridine hydrogen sulfate formed; in its absence the salt formed as a solid cake which was dissolved with difficulty during the titration.) After the reaction mixtures had returned to room temperature, they were titrated with Karl Fischer reagent. The average titers calculated to 4.5 ± 0.1 weight per cent water.

No interference was observed in the titration of sulfur with Fischer reagent. Two gram samples suspended in 25 ml. of methanol gave net titers equivalent to 0.05 ± 0.00 per cent moisture.[3]

CHAPTER VIII

Reactions of Karl Fischer Reagent with Inorganic Compounds

A. General

In the previous chapter it was demonstrated that the Karl Fischer reagent gave quantitative results in the determination of hydrate and free water in many inorganic compounds. However, interference from side reactions is frequently encountered and consequently a well-rounded knowledge of the nature and stoichiometry of these interfering reactions is essential to the successful use of the reagent.

A few investigators observed that certain types of inorganic compounds interfered. Zimmerman[1] correctly pointed out that the unmodified method cannot be used in the presence of strongly alkaline bases, ammonia, soda, and sodium perborate. His difficulty with sodium phosphate and pyrophosphate is hard to explain since these compounds, with the exception of the so-called trisodium phosphate, offer no interference. Almy, Griffin, and Wilcox[2] also indicated that the caustic alkalis interfered but further observed that in certain cases, when the inorganic hydroxide was known, a stoichiometric correction could be applied, permitting the calculation of the actual water content. They also reported that borates and boric acid gave erroneously high results, apparently through reaction with the methanol of the reagent.

In view of the well known oxidizing-reducing properties of an iodine–iodide couple, it was not surprising to find evidence of this action in Fischer reagent. If a metal ion was capable of existence in more than one valence state, then hydrogen iodide might reduce or iodine might oxidize the ion with a corresponding effect upon the apparent water titer. The gross reaction of an inorganic substance

---

[1] Zimmermann, A., *Fette u. Seifen,* **46,** 446–50 (1939).

[2] Almy, E. G., Griffin, W. C., and Wilcox, C. S., *Ind. Eng. Chem., Anal. Ed.,* **12,** 392–6 (1940).

with the Fischer reagent could involve both water formation and oxidation-reduction effects.

Although Karl Fischer reagent was always standardized by titration of solutions of known moisture content, this actually meant that the iodine concentration of the solution had been established. Therefore, the disappearance of iodine would be either a measure of water content or reducing ability in the sample. The oxidizing capacity of a material was equivalent to a corresponding increase in iodine content of the titrated solution.

A thorough investigation of the stoichiometry of these interfering reactions was made in the authors' laboratory.[3,4]

In making the analyses, unless otherwise noted, up to about 15 millimoles of the samples were transferred to glass-stoppered volumetric flasks, covered with 25 ml. of dry methanol, and titrated directly with Karl Fischer reagent. Except where noted in Table LXXXII, vigorous agitation was not necessary; the mixtures titrated immediately to permanent endpoints.

B. Reactions Involving Oxygen of Metal Oxides and Hydroxides

In addition to iodine, sulfur dioxide, and pyridine, a methanol solution of Karl Fischer reagent contained considerable amounts of the pyridine salts of hydriodic and methylsulfuric acids arising from initial water present in the components and from preparative side reactions. (These reactions are discussed in Chapter III.) The latter constituents, which were also the endproducts of the analytical reaction with water, ordinarily had no effect on the titration of water. However, hydriodic acid, even when buffered by pyridine, had a marked solvent action upon metal oxides and hydroxides, normally liberating 1 molecule of water for each oxygen atom attacked, either with or without complex ion formation, as exemplified by the reaction:

$$MO + 2\,HI \longrightarrow MI_2 + H_2O \tag{1}$$

where M represents a divalent metal. This reaction was demonstrated in a study of the behavior of zinc oxide.[3]

Thus, although the initial action of the reagent on oxides and hydroxides presumably involved the liberation of water with the free acid of the reagent, the net effect was analogous to that of the

---

[3] Mitchell, J., Jr., Smith, D. M., Ashby, E. C., and Bryant, W. M. D., *J. Am. Chem. Soc.*, 63, 2927–30 (1941)

[4] Unpublished results from the authors' laboratory.

reaction of water with Fischer reagent. (The salt-forming function of pyridine has been ignored in order to simplify formulation.)

$$H_2O + I_2 + SO_2 + CH_3OH \longrightarrow 2\,HI + HSO_4CH_3 \tag{2}$$

$$ZnO + I_2 + SO_2 + CH_3OH \longrightarrow ZnI_2 + HSO_4CH_3 \tag{3}$$

$$NaOH + I_2 + SO_2 + CH_3OH \longrightarrow NaI + HI + HSO_4CH_3 \tag{4}$$

The quantitative reaction of the Karl Fischer reagent with inorganic oxides and hydroxides was fairly general and, therefore, served as the basis of a method for their analysis. These compounds of the alkali and alkaline earth metals reacted without difficulty and the alkali salts of weak oxygen acids behaved like a mixture of the respective oxides, of which only the basic oxides usually reacted with the reagent. (For example, $Na_2SO_3 \rightleftarrows Na_2O + SO_2$). Oxides of less electropositive metals were less reactive, although cuprous, zinc, silver, and mercuric oxides were readily converted. Aluminum, nickel, cupric, and plumbous oxides were unreactive. Table LXXXII shows the results of titrating a variety of inorganic oxides with Karl Fischer reagent. Most of these compounds give a net of 1 mole of iodine reacted per mole of oxide taken.

TABLE LXXXII

Action of Karl Fischer Reagent on Oxides[a]

Oxide	Sample added, mM[b]	Iodine consumed, mM[b]	Apparent moles of water formed per mole of oxide
Calcium, CaO	5.12	5.19	1.01
Magnesium, MgO	10.96	10.87	0.99
Zinc, ZnO	5.96	5.96	1.00
Silver, Ag_2O	5.68	5.91	1.04
Mercuric (yellow), HgO	6.86	6.76	0.99
Mercuric (red), HgO	6.05	6.05	1.00
Cuprous, Cu_2O	11.88	11.97	1.01
Cupric (ppt.), CuO	14.21	0.00	0.00
Lead, PbO	6.15	0.9	0.15
Aluminum, $Al_2O_3 \cdot xH_2O$	0.30 g.	0.00	0.00
Iron, Fe_3O_4	4.20	3.79[c]	0.89
Iron, Fe_2O_3	5.42	4.33[c]	0.80
Nickel, NiO	7.32	0.36	0.05

[a] This table is an expansion of that previously reported.[3]

[b] Millimoles.

[c] Maximum values involving 4 hours continuous shaking with excess Fischer reagent.

Those oxides reacting stoichiometrically, analogously to equation (3), were soluble in the Fischer reagent at the endpoint. The nickel, iron, aluminum, and lead oxides were insoluble initially in the methanol and a solid phase remained throughout the titration. The lead oxides precipitated a yellow solid, which tended to obscure the endpoint. It was thought that this insoluble lead compound occluded some of the oxide, further discouraging the reaction.

A portion of this yellow solid was isolated. On recrystallization from hot water, lustrous golden crystals were precipitated, typical of PbI_2; iodine was liberated from acid solution.

Oxides in Table LXXXII which reacted incompletely were treated with glacial acetic acid at 60°C.[4] Only PbO dissolved. Subsequent titration with Fischer reagent gave a ratio of 0.997 moles of iodine reacted per mole of oxide. Nickel oxide partially dissolved and on titration gave a value of 0.35 mole of apparent water per mole of oxide. The iron and aluminum oxides were insoluble and gave no evidence of reaction. No advantage was gained by using $BF_3 \cdot 2CH_3COOH$ or dichloroacetic acid as reaction media.

The fact that cuprous oxide readily dissolved in the reagent while cupric oxide was practically insoluble has been of value in analyzing mechanical mixtures of the two oxides.[3] Known weights of each were dispersed in 25 ml. of dry methanol and excess Fischer reagent was added. After standing for 10 minutes the excess reagent was determined by back titration with standard water-in-methanol. The results, calculated from the net iodine reacted, are shown in Table LXXXIII.

TABLE LXXXIII

Determination of Cuprous Oxide in Presence of Cupric Oxide[3]

Oxide added, wt. %		Cuprous oxide found, wt. %
Cupric	Cuprous	
0.0	100.0	100.0
39.8	60.2	60.7
65.5	34.5	34.9
92.0	8.0	7.9

Since microcrystalline oxides occurring as minerals were often much less soluble than some of the less dense synthetic products,

TABLE LXXXIV

Reactions of Minerals with Karl Fischer Reagent[3]

Mineral	Minimum shaking time, hrs.	Oxide, millimoles		
		Calculated[a]	Found	Ratio
Cuprite	2	2.67	2.53	0.95
Zincite	48	6.73	5.37	0.80

[a] Calculated assuming 100 per cent purity.

selected crystals of cuprite (Cu_2O) and zincite (ZnO) were ground to pass a 200-mesh screen. Samples were dispersed in methanol and excess Fischer reagent was added. The mixtures were shaken mechanically for varying times and the excess reagent was determined by back titration with water-in-methanol. Results are shown in Table LXXXIV. The minerals reacted quite slowly but the reactions apparently went to completion; the zincite results probably indicated about 20 per cent of inert impurities. No advantage was gained on substituting glycol for methanol as dispersing agent.

The metal hydroxides, at least insofar as they were soluble, reacted quantitatively, analogously to equation (4). Since a mole of water was involved for each mole of hydroxyl group, the titer was a direct measure of the combined alkali and moisture contents. The same net titer was obtained when an excess of glacial acetic acid of known moisture content was added before titration:

$$NaOH + CH_3COOH \qquad NaOOCCH_3 + H_2O \tag{5}$$

It might be expected in general, especially where oxidation-reduction reactions were not involved, that preliminary solution of all types of inorganic compounds in an inert anhydrous acid would not affect the Fischer reagent titer for complete reaction. Use of an acid of known low water content (such as concentrated H_2SO_4) would require a correction to the Fischer reagent titer, which, however, could be calculated accurately.

Results obtained on a few metal hydroxides are recorded in Table LXXXV.

Although the reaction of metal hydroxides was quantitative, permitting suitable corrections to the total titer when the concentration of water was desired, the direct titration lacked sensitivity when only small amounts of water were involved. An interesting variation in the application of the Karl Fischer reagent was employed by Suter[5]

---

[5] Suter, H. R., *Ind. Eng. Chem., Anal. Ed.,* 19, 326–9 (1947).

for the determination of small quantities of water in sodium hydroxide. Free water (20 to 200 milligrams) was separated from the sample by azeotropic distillation with xylene, after which the distillate was titrated with Karl Fischer reagent. The distillation apparatus requirements were quite simple, consisting of an electric heater, a 500 ml. two-neck ₮ 24/40 flask, a short bent delivery tube (acting as an air condenser), and a 250 ml. Erlenmeyer flask as a receiver protected with a drying tube attached at a point just above its 100 ml. level.

TABLE LXXXV

Action of Karl Fischer Reagent on Alkaline Hydroxides[4]

Hydroxide	Sample, g.	Apparent water found, millimoles	Total hydroxyl,[a] millimoles	Apparent moles of water per mole of hydroxyl	Calculated free water,[b] wt. %
Sodium, NaOH	0.3862	9.57	9.50	1.01	0.3
Lithium, LiOH	0.3050	13.17	11.50	1.15	9.8
Potassium, KOH	0.5126	11.04	8.05	1.37	10.5
Barium, $Ba(OH)_2.8H_2O$	0.3423	10.54	1.11	9.50	43.7[c]

[a] By acidimetric titration.

[b] From net Fischer titration value after correction for total milliequivalents of base.

[c] Calculated for octahydrate: 45.6 wt. % of water.

In carrying out an analysis, up to 26 grams of sample, depending on the water content, was weighed quickly in a weighing bottle and transferred to an oven-dried mortar containing a portion of a 100 ml. volume of xylene. The sample was ground rapidly and transferred to the distilling flask, and was washed with the remaining volume of xylene—preferably delivered from a wash bottle. The initial distillation rate was relatively high to sweep out the surface moisture. After the distillate became clear, however, the heater was adjusted to maintain reflux in the short vertical leg of the delivery tube with a small amount of distillate. When the distillation was completed (about 4 hours), 50 ml. of dry methanol were added to form and maintain a homogeneous solution during the subsequent titration of the distillate to the dead-stop endpoint.

In 7 determinations on a sample of sodium hydroxide, Suter[5] reported a mean value of 0.236 per cent water with an average deviation of 0.011 per cent. The method also was applied successfully to the estimation of moisture in alkali silicates and other inorganic salts.

C. Reactions Involving Peroxy Compounds

The action of oxidizing compounds such as inorganic persalts and peroxides was interesting since some complications could be expected involving oxidation of the hydriodic acid present in the reagent and consequent formation of free iodine. Zimmerman[1] found, however, that hydrogen peroxide did not react with either hydriodic acid or iodine. He assumed that it reacted preferentially with the excess sulfur dioxide of the reagent, according to the reaction:

$$H_2O_2 + SO_2 \longrightarrow H_2SO_4 \qquad (6)$$

The normal course of the reaction of Fischer reagent with water involved oxidation of the sulfur dioxide to pyridine methyl sulfate (equations 5 and 6, Chapter III) which was not ionized. This factor permitted proof that equation (6) represented the course of the peroxide reaction.[3] Barium chloride solution was added, respectively, to a sample of aqueous hydrogen peroxide solution after titration of Karl Fischer reagent and to an equal amount of Fischer reagent treated with water only. A greatly increased amount of simple sulfate ion was found in the former as indicated by the precipitation of barium sulfate.

Since no iodine was involved in reaction (6), the Karl Fischer reagent could be used for the determination of water in the presence of hydrogen peroxide as demonstrated in Table LXXXVI.

TABLE LXXXVI

Fischer Titration for Water in Hydrogen Peroxide Solutions[1]

Peroxide, wt. %	Water found by Karl Fischer titration, wt. %	Total, wt. %
30.3	69.4	99.7
20.4	80.0	100.4
14.9	85.4	100.3
6.4	93.4	99.8
3.4	96.5	99.9

The metal peroxides and persalts apparently behaved similarly. Sodium peroxide was shown to follow a course analogous to reaction (6),[3] and, based on the results shown in Table LXXXVII, other peroxides apparently did not interfere.

TABLE LXXXVII

Reaction of Inorganic Peroxides with Karl Fischer Reagent[4]

Peroxide	Formula weight	Concentration	Apparent water found, mM/g.[b]	Remarks
Sodium, Na_2O_2	78.00	C.P.	0.08	$\asymp$ 3% free NaOH
Barium, BaO_2	169.36	C.P.	0.20	$\asymp$ 10% $Ba(OH)_2$
Potassium persulfate, $K_2S_2O_8$	270.31	C.P.	0.027	$\asymp$ 0.05% H_2O
Ammonium persulfate, $(NH_4)_2S_2O_8$	228.20	C.P.	0.197	$\asymp$ 0.35% H_2O
Sodium pyrophosphate, $Na_4P_2O_7 \cdot H_2O_2$	299.97	C.P.	0.48	$\asymp$ 0.74% H_2O
Sodium carbonate, $Na_2CO_3 \cdot H_2O_2$	140.02	C.P.	7.10	$\asymp$ Na_2CO_3[a]
Sodium perborate, $NaBO_2 \cdot 3H_2O \cdot H_2O_2$[c]	153.88	C.P.	32.1	$\asymp$ $NaBO_2$[a] + H_2O
Magnesium, MgO_2	56.32	50%	17.41	$\asymp$ 50% $Mg(OH)_2$
Zinc, ZnO_2	97.38	55%	8.84	$\asymp$ 40% $Zn(OH)_2$ + 5% ZnO
Calcium, CaO_2	72.08	60%	8.77	$\asymp$ 20% $Ca(OH)_2$ + 20% CaO

[a] See this chapter, section D.

[b] Millimoles per gram; 10 millimole sample used for analysis.

[c] 3.5 millimoles used for analysis.

On prolonged standing with methanol before titration, oxygen was lost by sodium peroxide and the titer with Fischer reagent was proportionately increased. This condition should be avoided.

The presence of free base in the sodium and barium peroxides was expected, and the Karl Fischer titer served as a measure of free sodium hydroxide and barium hydroxide, respectively. Small quantities of water were not unusual in the persulfates and pyrophosphate. The apparent water contents found in both sodium carbonate peroxide and sodium perborate were almost exactly equivalent to the known reactions of carbonate and water plus metaborate (to be explained later in this chapter). Magnesium, zinc, and calcium peroxides were obtained in the concentrations indicated in the table; the remainders were known to be mixtures of the respective hydroxides and oxides. Assuming no interferences from the peroxides and no free water, the analyses indicated that the magnesium peroxide contained only hydroxide as diluent; the zinc compound contained principally the hydroxide and the calcium compound contained approximately equal proportions of oxide and hydroxide.

Manganese dioxide, MnO_2, dispersed in methanol, titrated directly with Karl Fischer reagent according to the net reaction:[3]

$$MnO_2 + I_2 + 2\,SO_2 + 2\,CH_3OH \longrightarrow MnI_2 + 2\,HSO_4CH_3 \qquad (7)$$

Data for this compound and the lead oxides, PbO_2 and Pb_3O_4, are given in Table LXXXVIII.

TABLE LXXXVIII

Reactions of Manganese Dioxide and Lead Oxides with Fischer Reagent

Oxide	Sample added, mM[c]	Iodine consumed, mM[c]	Apparent moles of water formed per mole of oxide
Manganese, MnO_2[a]	9.73	10.59	1.09
Lead, PbO_2	6.53	3.27	0.50
Lead, Pb_3O_4	1.32	2.90[b]	2.20

[a] Previously reported.[3]

[b] Maximum value obtained after 4 hours continuous shaking with excess Karl Fischer reagent.

[c] Millimoles.

Since the c.p. manganese dioxide reacted readily, indicating 1 mole of iodine consumed per mole of oxide, it was felt desirable to study the behavior of the mineral, pyrolusite ($MnO_2 + 2\% \ H_2O$). This material was ground to pass a 200-mesh screen and a sample calculated to contain 3.64 millimoles of manganese dioxide was dispersed in methanol. Excess Karl Fischer reagent was added and the mixture was shaken for 6 hours. The excess reagent in the resulting homogeneous solution was back titrated with a standard water-in-methanol solution. After correction for 2 per cent water, the net Fischer reagent titer was equivalent to 3.64 millimoles, a molar ratio of 1.00.[3]

The brown lead dioxide, when titrated with Fischer reagent, initially turned gray and finally formed the yellow iodide.[4] Similar observations were made on the red lead. At the endpoints apparently some unreacted oxides remained, for in both cases the heterogeneous insoluble portions contained materials having the colors of the respective oxides. Samples of each oxide were dispersed in glacial acetic acid and heated at 60°C. The lead dioxide, PbO_2, was not completely soluble, but on titration with Fischer reagent the molar ratio of "water" found to oxide added was 1.05, indicating a net reaction analogous to that of manganese dioxide (equation 7):

$$PbO_2 + I_2 + 2\,SO_2 + 2\,CH_3OH \longrightarrow PbI_2 + 2\,HSO_4CH_3 \qquad (8)$$

The red oxide, Pb_3O_4, was completely soluble in hot acetic acid and, on titration with Fischer reagent, 3.05 moles of apparent water were found for each mole of oxide added. This oxide actually is a molecular combination of 2 PbO + PbO_2 and breaks down to these oxides on treatment with acids. The Karl Fischer reagent titer, therefore, was consistent with the individual action of the two oxides.

D. Reactions of Weak Acids and Oxides

Orthoboric acid reacted with the alcohol of Fischer reagent forming methyl borate:[2,6]

$$H_3BO_3 + 3\,CH_3OH \longrightarrow B(OCH_3)_3 + 3\,H_2O \qquad (9)$$

$$\text{or: } H_3BO_3 + 3\,I_2 + 3\,SO_2 + 6\,CH_3OH \longrightarrow 6\,HI + 3\,HSO_4CH_3 + B(OCH_3)_3 \qquad (10)$$

This reaction apparently was independent of the reaction medium employed, since 3 moles of water per mole of boric acid were found

---

[6] Mitchell, J., Jr., Smith, D. M., and Bryant, W. M. D., *J. Am. Chem. Soc.* 62, 4–6 (1940).

whether the diluent was methanol, acetic acid, or dioxane. A modified Fischer reagent employing ethanol in place of methanol likewise reacted in the same manner. In this case triethyl borate was formed.

Boric oxide reacted in a similar manner:[3]

$$B_2O_3 + 3\,I_2 + 3\,SO_2 + 9\,CH_3OH \longrightarrow 6\,HI + 3\,HSO_4CH_3 + 2\,B(OCH_3)_3 \quad (11)$$

Metaboric acid, HBO_2, was prepared in crystalline form by heating orthoboric acid with steam at 150°C. and 1 atmosphere pressure for 4 to 6 hours. (Based on phase diagrams by von Stackelberg, Quatram, and Dressel.[7]) The crystals were ground and titrated in isopropanol suspension. The reaction was slower than that of related compounds but followed the expected course:[3]

$$HBO_2 + 3\,CH_3OH \longrightarrow B(OCH_3)_3 + 2\,H_2O \quad (12)$$

$$: HBO_2 + 2\,I_2 + 2\,SO_2 + 5\,CH_3OH \longrightarrow 4\,HI + 2\,HSO_4CH_3 + B(OCH_3)_3 \quad (13)$$

Arsenious oxide dissolved very slowly; after 4 hours of continuous agitation in the presence of excess Fischer reagent, a small amount of the unreacted oxide still remained as a solid phase. The moles of apparent water found per mole of oxide indicated a limit of 3, the value predicted by the reaction:[3]

$$As_2O_3 + 3\,I_2 + 3\,SO_2 + 3\,CH_3OH \longrightarrow 2\,AsI_3 + 3\,HSO_4CH_3 \quad (14)$$

TABLE LXXXIX

Reaction of Boric Acid, and Boric and Arsenious Oxides with Karl Fischer Reagent[3,4]

Substance	Sample added, mM[c]	Iodine consumed, mM[c]	Apparent moles of water formed per mole of sample
H_3BO_3	2.99	8.93	2.99[a]
B_2O_3	2.50	7.50	3.00
HBO_2	3.33	6.62	1.99
As_2O_3	1.39	3.86	2.78[b]

[a] Values in acetic acid and dioxane diluent and with Karl Fischer reagent made from ethanol were 2.98, 2.99, and 3.00, respectively.

[b] Slow reaction, maximum value after 4 hours shaking with excess Fischer reagent.

[c] Millimoles.

---

[7] von Stackelberg, M., Quatram, F., and Dressel, J., *Z. Elektrochem.*, 43, 14–29 (1937).

No increase in reaction was observed when arsenious oxide was dispersed in glycol or hot acetic acid before titration. Experimental data for these substances are given in Table LXXXIX. The dry boric acid and oxides were titrated directly with Karl Fischer reagent, while arsenious oxide first was dispersed in methanol.

E. Reactions of Some Salts of Inorganic and Organic Acids

In general the weak acid salts of the alkali metals appeared to react with Karl Fischer reagent in the same manner as the oxides. The stoichiometries of the reactions of carbonates, bicarbonates, sulfites, and pyrosulfites are illustrated by the following equations:[3]

$$K_2CO_3 + I_2 + SO_2 + CH_3OH \longrightarrow 2\,KI + CO_2 + HSO_4CH_3 \tag{15}$$

$$KHCO_3 + I_2 + SO_2 + CH_3OH \longrightarrow KI + HI + CO_2 + HSO_4CH_3 \tag{16}$$

$$Na_2SO_3 + I_2 + CH_3OH \longrightarrow 2\,NaI + HSO_4CH_3 \tag{17}$$

$$Na_2S_2O_5 + I_2 + CH_3OH \longrightarrow 2\,NaI + SO_2 + HSO_4CH_3 \tag{18}$$

Experimental data on these and other salts are given in Table XC.

The normal and acid alkali salts of most strong acids were not affected by Karl Fischer reagent, any titer being derived exclusively from free water or water of hydration as indicated in Chapter VII. The same was true of the related salts, sodium hyposulfite, $Na_2S_2O_6 \cdot 2H_2O$, and potassium pyrosulfate, $K_2S_2O_7$. Fischer's surprising observation that calcium carbonate did not interfere[8] was verified in the authors' laboratory. (Fischer reported a value of 0.23 per cent water found, compared with 0.24 per cent by weight loss at 120°C.) Sodium thiosulfate reacted with iodine in the usual way:[8]

$$2\,Na_2S_2O_3 + I_2 \longrightarrow 2\,NaI + Na_2S_4O_6 \tag{19}$$

The tetraborate and metaarsenite behaved like a mixture of oxides where the acidic oxide also was attacked (Table LXXXIX):[3]

$$Na_2B_4O_7 + 7\,I_2 + 7\,SO_2 + 19\,CH_3OH \longrightarrow 2\,NaI + 12\,HI + 7\,HSO_4CH_3 + 4\,B(OCH_3)_3 \tag{20}$$

$$(Na_2B_4O_7 \rightleftharpoons Na_2O + 2\,B_2O_3)$$

$$NaAsO_2 + 2\,I_2 + 2\,SO_2 + 2\,CH_3OH \longrightarrow NaI + AsI_3 + 2\,HSO_4CH_3 \tag{21}$$

$$(2\,NaAsO_2 \rightleftharpoons Na_2O + As_2O_3)$$

Among the compounds whose anions underwent oxidation or reduction reactions, sodium arsenate heptahydrate was found to behave in the following manner:[3]

---

[8] Fischer, K., *Angew. Chem.*, **48**, 394–6 (1935).

TABLE XC. Reaction of Various Salts with Karl Fischer Reagent[3,4]

Salt	Sample added, millimoles	Apparent water found, millimoles		Ratio (molar) of net apparent water to anhydrous salt
		Gross	Net[a]	
Salts which reacted stoichiometrically				
K_2CO_3	13.60	13.82		1.02
$KHCO_3$	16.12	16.22		1.01
Na_2SO_3	5.30	5.27		0.99
$Na_2S_2O_5$[b]	10.2	9.2		0.89
$K_2S_2O_5$[b]	5.24	4.61		0.88
$Na_2S_2O_3 \cdot 5H_2O$	2.12	11.68	1.08	0.51
$Na_2B_4O_7 \cdot 5H_2O$	0.393	4.76	2.79	7.10
$NaAsO_2$	3.21	6.23		1.94
$Na_2HAsO_4 \cdot 7H_2O$	3.38	33.54	9.95	2.94
$CuSO_4 \cdot 5H_2O$	5.07	22.70	−2.65	−0.52
$CuCl_2 \cdot 2H_2O$	3.55	5.34	−1.76	−0.50
$Cu(OOCCH_3)_2 \cdot H_2O$[c]	3.68	1.93	−1.75	−0.49
$SnCl_2 \cdot 2H_2O$	4.61	13.64	4.42	0.96
$NaNO_2$	9.94	4.93		0.50
$FeCl_3 \cdot 6H_2O$	1.96	11.00	−0.77	−0.39
Salts which reacted variably or for which no reaction mechanism was apparent				
$Na_2CrO_4 \cdot 4H_2O$	1.25	8.90	3.90	3.12
$Mg(NH_4)_2(CrO_4)_2 \cdot 6H_2O$	2.30	19.92	6.12	2.66
$Na_2Cr_2O_7 \cdot 2H_2O$	0.69	2.82	1.34	1.94
$K_2Cr_2O_7$	1.16	1.69		1.46
$(NH_4)_2Cr_2O_7$	2.02	4.65		2.30
$Na_2S \cdot 9H_2O$	2.02	20.05	1.75	0.87
$Na_3PO_4 \cdot 12H_2O$	0.68	7.36	−0.67	
$20MoO_3 \cdot 2H_3PO_4 \cdot 48H_2O$	0.29	14.80[d]		
$NH_4[Cr(NH_3)_2(SCN)_4] \cdot H_2O$	14.13	8.98[e]		
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	3.48	24.21	10.29	2.96
$ZrOCl_2 \cdot 8H_2O$	1.93	15.79	0.34	0.18[f]
	0.77	6.72	0.53	0.69[f]
$ZrO(NO_3)_2 \cdot 2H_2O$	7.00	27.74	13.74	1.96
$Bi_2O_3 \cdot CO_2 \cdot H_2O$	1.52	0.19		
$Al_2O(OOCCH_3)_2 \cdot 4H_2O$[g]	2.10	1.33		

---

[a] Net millimoles of water after correction for known water of hydration.

[b] This compound apparently was not pure.

[c] This cupric salt of an organic acid was included for comparison with the corresponding inorganic salts.

[d] Phosphomolybdic acid: calculates as 51 moles of water per mole of salt.

[e] Reinecke salt: calculates as 0.64 mole of water per mole of salt.

[f] Analyses on two different lots.

[g] Basic aluminum acetate.

$$Na_2HAsO_4 \cdot 7\,H_2O + 10\,I_2 + 11\,SO_2 + 11\,CH_3OH \longrightarrow 2\,NaI + 15\,HI + AsI_3 + 11\,HSO_4CH_3 \quad (22)$$

$$(2\,Na_2HAsO_4 \rightleftharpoons 2\,Na_2O + H_2O + As_2O_5)$$

$$As_2O_5 + 3\,I_2 + 5\,SO_2 + 5\,CH_3OH \longrightarrow 2\,AsI_3 + 5\,HSO_4CH_3 \quad (23)$$

Sodium nitrite was another example of a compound reacting stoichiometrically as follows:[3]

$$NaNO_2 + 0.5\,I_2 + SO_2 + CH_3OH \longrightarrow NaI + NO + HSO_4CH_3 \quad (24)$$

This reaction was relatively slow and apparently permitted the determination of small quantities of free water.[4] Duplicate values of 0.15 ± 0.00 per cent water were found in the sample. (Identical results were obtained on titration at the reduced temperature of O°C.) Continued titration gave values of 0.49 ± 0.01 mole of iodine consumed per mole of compound.[4] Prior treatment of the sodium nitrite with sodium acetate and acetic acid led to a reduced reaction.* A 0.6 gram sample of dry salt, treated for 1 hour at room temperature with 1 gram of dry sodium acetate and 25 ml. of glacial acetic acid, on titration with Fischer reagent gave a net reaction of 0.15 mole of apparent water per mole of sodium nitrite.[4]

If an oxidizable or reducible metal ion was present, the reaction included reduction or oxidation of the cation.

Cupric salts exemplified this type of reaction:[3]

$$CuCl_2 \cdot 2\,H_2O + 1.5\,I_2 + 2\,SO_2 + 2\,CH_3OH \longrightarrow CuI + 2\,HCl + 2\,HI + 2\,HSO_4CH_3 \quad (25)$$

Karl Fischer,[8] in establishing the possible application of his reagent to salt hydrates, erroneously concluded that only 4 of the 5 water molecules of cupric sulfate pentahydrate were titrated. Since $CuSO_4 \cdot H_2O$ was formed on drying at 120°C. and the anhydrous salt was obtained only at temperatures above 200°C., Fischer assumed that the water in the monohydrate was too tightly bound to be titrable. Experiments in the authors' laboratory with the pentahydrate[3] indicated that all of the water was titrated but that the simultaneous reduction of the metal ion liberated 0.5 mole of iodine. The net reaction, therefore, indicated 4.5 moles of water instead of 5:

---

* This technique was used by Raschig to eliminate nitrous acid interference in the idiometric determination of sulfur dioxide. In aqueous solution the sodium acetate-acetic acid had a buffering effect (see Chapter III).

$$CuSO_4{\cdot}5\,H_2O + 5\,I_2 + 5\,SO_2 + 5\,CH_3OH \longrightarrow CuSO_4 + 10\,HI + 5\,HSO_4CH_4 \quad (26)$$

$$CuSO_4 + 2\,HI \longrightarrow CuI + 0.5\,I_2 + H_2SO_4 \quad (27)$$

These combined yielded:

$$CuSO_4{\cdot}5\,H_2O + 4.5\,I_2 + 5\,SO_2 + 5\,CH_3OH \longrightarrow CuI + 8\,HI + 5\,HSO_4CH_3 + H_2SO_4 \quad (28)$$

Semiquantitative confirmation of the above mechanism was obtained when anhydrous cupric sulfate was placed in Fischer reagent which had been exactly pretitrated. After standing for 1 hour, the liberated iodine was titrated with standard water-in-methanol. A value of 0.4 mole of iodine per mole of cupric salt was found.[4] The behavior of cupric acetate monohydrate likewise indicated a reaction of the type indicated in equation (25).

The oxidation of additional iodine was apparent in the reactions involving stannous chloride:[3]

$$SnCl_2{\cdot}2\,H_2O + 2\,I_2 + 2\,SO_2 + 2\,CH_3OH \longrightarrow SnCl_2 + 4\,HI + 2\,HSO_4CH_3 \quad (29)$$

$$SnCl_2 + I_2 + 2\,HI \longrightarrow SnI_4 + 2\,HCl^* \quad (30)$$

The net reaction was represented by:

$$SnCl_2{\cdot}2H_2O + 3\,I_2 + 2\,SO_2 + 2\,CH_3OH \longrightarrow SnI_4 + 2\,HCl + 2\,HI + 2\,HSO_4CH_3 \quad (31)$$

Ferric chloride hexahydrate gave a net titer equivalent to 5.61 moles of water per mole of salt. This probably was evidence of reduction to ferrous salt[3]:

$$FeCl_3{\cdot}6\,H_2O + 5.5\,I_2 + 6\,SO_2 + 6\,CH_3OH \longrightarrow FeI_2 + 9\,HI + 3\,HCl + 6\,HSO_4CH_3 \quad (32)$$

The chromates and dichromates reacted to a variable extent, either at room temperature or —40°C. Sodium chromate tetrahydrate in methanol reacted slowly, the color of the solution turning from yellow to green during the addition of Karl Fischer reagent. When shaken for 1 hour with excess Fischer reagent and after correction for the water of hydration, approximately 3 moles of iodine were consumed per mole of the chromate. The dichromates behaved in a similar manner, although the interfering reaction was considerably slower than with the chromates. Up to 80 milligrams

---

* Possibly the actual course of this reaction was $SnCl_2 + I_2 \longrightarrow SnCl_2I_2$. In either case 1 mole of iodine would be consumed per mole of compound.

of water added to the anhydrous potassium dichromate could be titrated quantitatively. On shaking for 1 hour with excess Fischer reagent, however, between 1.5 and 2 moles of iodine were consumed per mole of dichromate. Potassium permanganate dispersed in methanol or pyridine gave only a slight reaction (0.05 mole water per mole $\Bumpeq$ 0.57 per cent water) even after shaking for 1 hour at room temperature.[4]

Sodium sulfide was oxidized to a variable extent by the iodine of the reagent.[3] During titration of the nonahydrate, sulfur was first precipitated.

$$Na_2S + I_2 \longrightarrow 2\,NaI + S \tag{33}$$

The sulfur apparently reacted to some extent with the hydriodic acid, in accordance with the well-known reversible reactions:

$$H_2S + I_2 \rightleftharpoons 2\,HI + S \tag{34}$$

For the hydrate, $Na_2S{\cdot}9H_2O$, values have been observed varying from 9.75 to 9.89 moles of apparent water per mole of hydrate after titration at room temperature or at —40°C. Milberger and his coworkers[9] found that addition of excess Fischer reagent to approximately 6 milligram quantities of dry hydrogen sulfide in methanol solution, followed by back titration with standard water-in-methanol, gave molar ratios of iodine consumed to sulfide added of about 1 : 1.

Although the primary and secondary sodium orthophosphates gave no evidence of interfering reactions, the so-called trisodium phosphate dodecahydrate gave a molar ratio of water found to hydrate added of 10.85. A sample dried to constant weight at 110°C. (weight loss $\Bumpeq$ 10.80 moles of water per mole of salt) gave a titer with Fischer reagent equivalent to about 5 per cent as sodium hydroxide, qualitative evidence that the free base was present in the hydrate.[4]

The degrees of hydration of phosphomolybdic acid, Reinecke salt, ammonium molybdate, and zirconyl chloride and nitrate were not known exactly, and the hydrates in most cases were thermally unstable so that no conclusions appeared justified regarding the reactions with Fischer reagent. The values for zirconyl chloride did

---

[9] Milberger, E. C., Uhrig, K., Becker, H. C., and Levin, H., paper presented before the Division of Analytical and Microchemistry, 110th meeting of the American Chemical Society, Chicago, Ill., September 10, 1946.

not indicate definite interference; however, the corresponding figure for the nitrate, which was twice that of the presumed dihydrate, was not readily explained.

Bismuth subcarbonate was insoluble, possibly accounting for the negligible reaction. A value of 4 moles of iodine consumed per mole of salt might be expected [based on reaction (14)]. The particular sample used was fresh C.P. material; the presence of water of hydration was not verified.

In the presence of aluminum chloride, somewhat erratic results were obtained for the titration of water with Karl Fischer reagent. A sample of the hexahydrate indicated quantitative recovery (6.02 moles of water found per mole of hydrate added).[4] About 1 g. of anhydrous salt in 25 ml. of acetic acid containing 51.9 mg. of water gave a recovery of 47 mg. of water; in dioxane containing 83.6 and 166 milligrams of water, 88.6 and 160 milligrams were found, respectively.[4] The low result in Table XC for basic aluminum acetate may have been due to its insolubility in methanol and Fischer reagent. No increase in apparent water was observed after excess Fischer reagent was added and the sample was placed on a mechanical shaker for 30 min. before back titration with standard water-in-methanol. The apparent low recovery was also evident when the compound was dispersed in glacial acetic acid, dichloroacetic acid and acetic acid containing about 100 grams of boron trifluoride per liter of solution. The basic aluminum acetate was almost completely soluble in concentrated sulfuric acid. A 0.6 gram sample was weighed into a 250 ml. volumetric flask and 5 ml. of sulfuric acid was added. The mixture was heated at 60°C. for 20 minutes, then allowed to cool to about 5°C. in an ice bath. Twenty milliliters of 1 : 1 pyridine–dioxane were added carefully and the final solution was titrated with Fischer reagent. Approximately 2.8 moles of apparent water were found per mole of compound[4]; on the basis of the formula, $Al_2O(OOCCH_3)_4 \cdot 4H_2O$, at least 4 moles would be expected.

F. Miscellaneous Reactions

Hydroxylamine hydrochloride initially reduced the iodine of Fischer reagent, possibly according to the reaction:[4]

$$2\,H_2NOH + 3\,I_2 + 2\,SO_2 + 2\,CH_3OH \longrightarrow 6\,HI + 2\,HSO_4CH_3 + N_2 \quad (35)$$

As soon as sufficient sulfur dioxide (from Fischer reagent) was present, however, the preferential sulfamate reaction occurred:

$$NH_2OH + SO_2 + C_5H_5N \longrightarrow C_5H_5N{\cdot}SO_3H{\cdot}NH_2 \qquad (36)$$

Confirmation of this reaction was obtained by isolation of the crystalline material which was identified as pyridine sulfamate by its optical crystallographic constants.[4]

Reaction (36) did not involve iodine and consequently only partial and variable net reaction with iodine was observed. Reaction (35) was completely inhibited by treatment of the hydroxylamine salt with excess of a 0.5 *M* solution of sulfur dioxide in pyridine or spent Fischer reagent[4]; the net reaction after titration with unspent reagent was then a measure of free moisture.

Solutions containing ammonia could not be titrated directly, possibly because of interfering reactions involving the formation of nitrogen iodides. Preliminary reaction with glacial acetic acid was sufficient to inhibit the interference:[4]

$$NH_3 + CH_3COOH \longrightarrow CH_3COONH_4 \qquad (37)$$

Approximately 2 gram samples of aqueous ammonium hydroxide were weighed into individual 100 ml. glass-stoppered volumetric flasks containing about 30 ml. of dry methanol. These solutions were diluted to the mark with additional methanol. Duplicate 10 ml. aliquots were transferred to 250 ml. volumetric flasks, 5 ml. of glacial acetic acid added, and the solutions titrated with Karl Fischer reagent. After correction for the water in 9.7 ml. of the same methanol (approximate volume in 10 ml. aliquot) plus 5 ml. of acetic acid, the net titers were equivalent to 72.0 ± 0.1 per cent water. Separate analyses by acidimetric titration to methyl red gave values of 28.0 ± 0.0 per cent ammonia in these solutions.[4]

Anhydrous ammonia, because of the large samples required, was analyzed most conveniently for moisture by use of the apparatus and procedure for condensable gases (Fig. 31, Chapter VI). In this case after most of the ammonia had evaporated and the methanol solution had warmed to room temperature, 5 ml. of acetic acid were added just before titration with Fischer reagent. Duplicate samples of anhydrous ammonia analyzed 47 ± 3 ppm.[4]

PART TWO

Organic Reactions Involving Liberation or Consumption of Water. Application of Karl Fischer Reagent to Quantitative Determination of Organic Functional Groups

CHAPTER IX

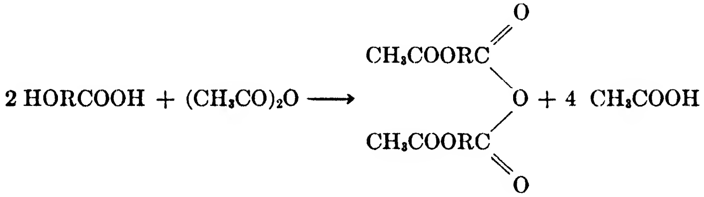
Determination of Alcoholic Hydroxyl

A. Methods of Analysis for Alcoholic Hydroxyl

One of the earliest chemical methods for the determination of alcoholic hydroxyl was that proposed by Benedikt and Ulzer[1] employing acetic anhydride for the analysis of higher hydroxylated fatty acids:

$$\mathrm{HORCOOH + (CH_3CO)_2O \longrightarrow CH_3COORCOOH + CH_3COOH} \qquad (1)$$

The ester was isolated and determined by saponification equivalent. However, Lewkowitsch[2] demonstrated that in such cases some dehydration of the fatty acids occurred and the resulting ester–anhydride mixtures were not hydrolyzed completely even with excess hot water.



(2)

Consequently the saponification value was always in error. He proposed a modified technique in which the acetylation product was isolated and saponified with a known quantity of caustic, after which sulfuric acid exactly equivalent to the caustic was added. The acetic acid formed on hydrolysis (equivalent to original hydroxyl) was determined either by distillation or by extraction from the higher fatty acids. The acetic anhydride technique was improved considerably by Verley and Bölsing,[3] who introduced pyridine as a catalyst both in the acetylation and in a subsequent anhydride hydrolysis step:

---

[1] Benedikt, R., and Ulzer, F., *Chem.-Ztg.*, **18**, 468–70 (1887).
[2] Lewkowitsch, J., *J. Soc. Chem. Ind.*, **9**, 842–8 (1890); **16**, 503–6 (1897).
[3] Verley, A., and Bölsing, F., *Ber.*, **34**, 3354–8 (1901).

$$ROH + (CH_3CO)_2O \longrightarrow CH_3COOH + CH_3COOR \qquad (3a)$$

$$H_2O + (CH_3CO)_2O \longrightarrow 2\,CH_3COOH \qquad (3b)$$

The net decrease in titrable acid between a blank and the sample then became a measure of alcoholic hydroxyl. The procedure of Verley and Bölsing, usually with some modification, has been the basis for the analysis of a wide variety of hydroxylated materials including fats,[4-7] hydroxylated fatty acids,[8,9] oils,[10,11] sugars,[12-14] cellulose derivatives,[15] mono- and polyhydric alcohols,[16-21] menthol,[22,23] and naphthol.[24] General micro methods have been proposed.[25,26] The combined esterification-saponification technique[1] had one advantage. In the absence of other saponifiable material the acetate ester, formed on reaction with anhydride, in some cases could be isolated, weighed, and saponified—permitting an estimate of the equivalent weight of the hydroxylated compound.[27] A reagent of acetic anhydride containing 5 per cent acetyl chloride has also been reported,[28] while some workers used benzoic[29] and phthalic

[4] André, E., *Bull. soc. chim.*, (4), **37**, 335–9 (1925).

[5] West, E. S., Hoagland, C. L., and Curtis, G. H., *J. Biol. Chem.*, **104**, 627–34 (1934).

[6] Kaufmann, H. P., *Fette u. Seifen*, **4**, 150–3 (1937).

[7] Norman, W., and Schildknecht, E., *Fettchem. Umschau*, **40**, 194–7 (1933).

[8] Hafner, P. G., Swinney, R. H., and West, E. S., *J. Biol. Chem.*, **116**, 691–7 (1936).

[9] Ogg, C. L., Porter, W. L., and Willits, C. D., *Ind. Eng. Chem., Anal. Ed.*, **17**, 394–7 (1945).

[10] Delaby, R., and Sabetay, S., *Bull. soc. chim.*, (5), **2**, 1716–24 (1935).

[11] Marks, S., and Morrell, R. S., *Analyst*, **56**, 428–9 (1931).

[12] Petersen, V. L., and West, E. S., *J. Biol. Chem.*, **74**, 379–83 (1927).

[13] Leman, A., *Compt. rend.*, **214**, 84–7 (1942); *Bull. soc. chim.*, (5), **10**, 235 (1943).

[14] Freed, M., and Wynne, A. M., *Ind. Eng. Chem., Anal. Ed.*, **8**, 278–9 (1936).

[15] Malm, C. J., Genung, L. B., and Williams, R. F., Jr., *Ibid.*, **14**, 935–40 (1942).

[16] Pohle, W. D., and Mehlenbacher, V. C., *Oil & Soap*, **23**, 48–50 (1946).

[17] Wilson, H. C., and Hughes, W. C., *J. Soc. Chem. Ind.*, **58**, 74–7 (1939).

[18] Shaefer, W. E., *Ind. Eng. Chem., Anal. Ed.*, **9**, 449–50 (1937).

[19] Moore, J. C., and Blank, E., *Oil & Soap*, **20**, 178 (1943).

[20] Poznanski, S., *J. Am. Chem. Soc.*, **50**, 981–8 (1928).

[21] Sapgir, I. N., and Frolova, R. A., *Sintet. Kauchuk*, **5**, 17–9 (1936).

[22] Brignall, T. W., *Ind. Eng. Chem., Anal. Ed.*, **13**, 166–9 (1941).

[23] Jones, J. S., and Fang, S. C., *Ibid.*, **18**, 130–1 (1946).

[24] Leman, A., *Compt. rend.*, **202**, 579–81 (1936); *Ann. chim.*, **9**, 357–446 (1938).

[25] Stodola, F. H., *Mikrochemie*, **21**, 180–3 (1937).

[26] Petersen, J. W., Hedberg, K. W., and Christensen, B. E., *Ind. Eng. Chem., Anal. Ed.*, **15**, 225–6 (1943).

[27] Steiner, C., *Chem.-Ztg.*, **59**, 795–6 (1935).

[28] Verley, A., *Bull. soc. chim.*, (4), **43**, 469–72 (1928).

[29] Leman, A., *Compt. rend.*, **205**, 357–9 (1939); *Bull. soc. chim.*, (5), **7**, 105–113 (1940).

anhydrides.[30,30a] Elving and Warshowsky[30a] found that phthalic anhydride could be used to advantage in the determination of primary and some secondary alcohols in the presence of aldehydes, water and phenols.

The action of acetyl chloride, the most commonly employed acyl halide, with alcohols was analogous to that of anhydrides but, because of its greater activity, required less drastic conditions to effect esterification.

$$\mathrm{ROH} + \mathrm{CH_3COCl} \longrightarrow \mathrm{CH_3COOR} + \mathrm{HCl} \tag{4}$$

$$\mathrm{H_2O} + \mathrm{CH_3COCl} \longrightarrow \mathrm{CH_3COOH} + \mathrm{HCl} \tag{5}$$

Adam[31] first reported using this reagent but it did not receive much attention until Smith and Bryant[32] introduced the use of pyridine as an essential part of the procedure. The resulting acetyl pyridinium chloride so reduced the vapor pressure of the active ingredient that it could be handled at room temperature without any significant volatilization losses. Other investigators have used this general reagent in the analysis of fats[33] and distilled spirits.[34] Acetyl chloride also has been found applicable on a micro scale.[35,36] In some cases lauroyl chloride[37] and stearoyl chloride[38] have been used with success.

All of these differential titration methods were lacking in sensitivity when the sample contained considerable amounts of water, acids, or easily hydrolyzed esters. Water, for example, reacted mole for mole with the reagents at a faster rate than did the alcohols. Acids proportionately increased the titration with base and, if present in excess, introduced relatively large corrections. Some of the lower esters could be hydrolyzed as the titration approached neutrality, thereby consuming caustic. Furthermore, these procedures were limited to the analysis of primary or secondary alcohols; ter-

---

[30] Glichitch, L. S., and Naves, Y. R., *Chimie & industrie*, **29**, 1024–8 (1933).

[30a] Elving, P. J., and Warshowsky, B., *Ind. Eng. Chem., Anal. Ed.*, **19**, 1006–10 (1947).

[31] Adam, F., *Chem. Zentr.*, **1899**, I, 1226–7.

[32] Smith, D. M., and Bryant, W. M. D., *J. Am. Chem. Soc.*, **57**, 61–5 (1935).

[33] Kaufmann, H. P., and Funke, S., *Ber.*, **70**, 2549–54 (1937).

[34] Schicktanz, S. T., and Etienne, A. D., *Ind. Eng. Chem., Anal. Ed.*, **11**, 390–3 (1939).

[35] Christensen, B. E., Pennington, L., and Dimick, P. K., *Ibid.*, **13**, 821–3 (1941).

[36] Christensen, B. E., and Pennington, L., *Ibid.*, **14**, 54–5 (1942).

[37] Grün, A., and Wirth, T., *Z. deut. Öl- u. Fett-Ind.*, **41**, 145–7 (1921).

[38] Raymond, E., and Bouvetier, E., *Compt. rend.*, **209**, 439–41 (1939).

tiary alcohols were partially dehydrated to olefins and consequently gave consistently low results. On the other hand, these methods were generally applicable to the determination of phenolic hydroxyl.

Other reported methods of limited application involved the use of the Grignard reagent:[39–44]

$$CH_3MgI + 2\,ROH \longrightarrow CH_4 + Mg(OR)_2 + HI \qquad (6)$$

xanthation:[45]

$$ROH + CS_2 + NaOH \longrightarrow ROC(S)SNa + H_2O \qquad (7)$$

$$2\,ROC(S)SNa + I_2 \longrightarrow 2\,NaI + ROC(S)S{\cdot}SC(S)OR \qquad (8)$$

triphenylchloromethane (trityl chloride):[46]

$$ROH + (C_6H_5)_3CCl \longrightarrow (C_6H_5)_3COR + HCl \qquad (9)$$

This reaction was almost specific for primary alcohols. Up to 95 per cent of primary was recovered in the presence of secondary and tertiary alcohols.[46]

bromination for phenols:[47–50]

$$C_6H_5OH + 3\,Br_2 \longrightarrow HOC_6H_2(Br)_3 + 3\,HBr \qquad (10)$$

sodamide:[51,52]

$$NaNH_2 + ROH \longrightarrow NaOR + NH_3 \qquad (11)$$

sulfonation:[27]

$$ROH + H_2SO_4 \longrightarrow ROSO_3H + H_2O \qquad (12)$$

hydriodic acid:[53,54]

$$ROH + HI \longrightarrow RI + H_2O \qquad (13)$$

[39] Zerewitinoff, T., *Ber.*, **40**, 2023–31 (1907); *Z. anal. Chem.*, **52**, 729–37 (1913).
[40] Williams, R. J., *J. Am. Chem. Soc.*, **58**, 1819–20 (1936).
[41] Braude, E. A., and Stern, E. S., *J. Chem. Soc.*, **1946**, 404–6.
[42] Tschugaeff, L., *Ber.*, **35**, 3912–4 (1902).
[43] Hibbert, H., and Sudborough, J. J., *J. Chem. Soc.*, **85**, 933–8 (1904).
[44] Hollyday, W., and Cottle, D. L., *Ind. Eng. Chem., Anal. Ed.*, **14**, 774–6 (1942).
[45] Sermais, B., *Rev. gén. mat. plastiques*, **12**, 165, 167–9 (1936).
[46] Sabetay, S., *Compt. rend.*, **203**, 1164–6 (1936).
[47] Koppeschaar, W. F., *Z. anal. Chem.*, **15**, 233–45 (1876).
[48] Day, A. R., and Taggart, W. T., *Ind. Eng. Chem.*, **20**, 545–7 (1928).
[49] Kaplan, D., *Chemistry & Industry*, **1944**, 244–5.
[50] Bielenberg, W., and Kuhn, W., *Z. anal. Chem.*, **126**, 88–94 (1943).
[51] Schryver, S. B., *J. Soc. Chem. Ind.*, **18**, 553–6 (1899).
[52] Naves, Y. R., *Helv. Chim. Acta*, **28**, 278–83 (1945).
[53] Prunier, L., *Z. anal. Chem.*, **35**, 218–20 (1896).
[54] Mitchell, H. K., and Williams, R. J., *J. Am. Chem. Soc.*, **60**, 273–6 (1938).

and nitrous acid:[55, 56]

$$ROH + HNO_2 \longrightarrow RNO_2 + H_2O \qquad (14)$$

$$RNO_2 + H_2O \xrightarrow{HCl} HNO_2 + ROH \qquad (15)$$

$$HNO_2 + 2\,HI \longrightarrow I_2 + NO + H_2O \qquad (16)$$

Oxidation methods have been described employing potassium permanganate,[57,58] chromic acid,[59] and acid dichromate.[60-71] For the analysis of polyhydric alcohols, in which at least two of the hydroxyls were adjacent, periodic acid[72-77] and lead tetraacetate[78,79] were widely used as specific reagents.

Nieuwland and his coworkers[80-82] demonstrated that boron trifluoride was an effective esterification catalyst. This was made the basis of a new titrimetric procedure for alcoholic hydroxyl, independent of acidimetry, when Bryant, Mitchell, and Smith[83] found

---

[55] Fischer, W. M., and Schmidt, A., *Ber.*, **B57**, 693–8 (1924); **B59**, 679–82 (1926).

[56] Skrabal, R., *Z. anal. Chem.*, **119**, 222–6 (1940).

[57] Herzog, R. D., *Ann.*, **351**, 263–6 (1907).

[58] Barendrecht, H. P., *Z. anal. Chem.*, **52**, 167–72 (1913).

[59] Semichon, L., and Flanzy, M., *Compt. rend.*, **195**, 254–6 (1932).

[60] Landsberg, G., *Z. physiol. Chem.*, **41**, 505–25 (1904).

[61] Chabot, G., *Bull. soc. chim. Belg.*, **34**, 328–37 (1925).

[62] Johnson, M. J., *Ind. Eng. Chem., Anal. Ed.*, **4**, 20–2 (1932).

[63] Werkmann, C. H., and Osburn, O. L., *Ibid.*, **3**, 387–8 (1931).

[64] Kozelka, F. L., and Hine, C. H., *Ibid.*, **13**, 905–7 (1941).

[65] Shatalov, V. P., and Lapshinov, I. I., *Sintet. Kauchuk*, **5**, 38–40 (1936).

[66] Thorpe, T. E., and Holmes, J., *J. Chem. Soc.*, **85**, 1–6 (1904).

[67] Boorman, E. J., *Analyst*, **64**, 791–4 (1939).

[68] Benedict, F. G., and Norris, R. S., *J. Am. Chem. Soc.*, **20**, 293–302 (1898).

[69] Kuriloff, B., *Ber.*, **30**, 741–3 (1897).

[70] Durodie, J., and Roelens, E., *Bull. soc. chim.*, (5), **10**, 169–76 (1943).

[71] Alekseeva, M. V., *J. Gen. Chem. USSR*, **5**, 1324–30 (1935).

[72] Malaprade, L., *Compt. rend.*, **186**, 382–4 (1928); *Bull. soc. chim.*, (4), **43**, 683–96 (1928); *Bull. soc. chim.*, (5), **1**, 833–52 (1934).

[73] Fleury, P., and Lange, J., *J. pharm. chim.*, **17**, 196–208 (1933).

[74] Allen, N., Charbonnier, H. Y., and Coleman, R. M., *Ind. Eng. Chem., Anal. Ed.*, **12**, 384–7 (1940).

[75] Price, C. C., and Kroll, H., *J. Am. Chem. Soc.*, **60**, 2726–9 (1938).

[76] Jackson, E. L., and Hudson, C. S., *Ibid.*, **59**, 994–1003, 2049–50 (1937); **61**, 959–60, 1530–2 (1939).

[77] Reinke, R. C., and Luce, E. N., *Ind. Eng. Chem., Anal. Ed.*, **18**, 244–5 (1946).

[78] Criegee, R., *Ber.*, **64**, 260–6 (1931).

[79] Criegee, R., and Büchner, E., *Ibid.*, **73**, 563–75 (1940).

[80] Bowlus, H., and Nieuwland, J. A., *J. Am. Chem. Soc.*, **53**, 3835–40 (1931).

[81] Hinton, H. D., and Nieuwland, J. A., *Ibid.*, **54**, 2017–8 (1932).

[82] Hennion, G. F., Hinton, H. D., and Nieuwland, J. A., *Ibid.*, **55**, 2857–60 (1933).

[83] Bryant, W. M. D., Mitchell, J., Jr., and Smith, D. M., *Ibid.*, **62**, 1–3 (1940).

that by employing a large excess of acetic acid with boron trifluoride as catalyst the acid–ester equilibrium could be shifted almost completely in favor of water and ester:

$$ROH + CH_3COOH \xrightarrow{BF_3} CH_3COOR + H_2O \qquad (17)$$

The water formed, which was equivalent to hydroxyl, was determined by direct titration with Karl Fischer reagent. This procedure was generally applicable to all types of primary, secondary, and tertiary aliphatic and alicyclic alcohols (including branched chain types and hydroxy acids), as well as such aromatic alcohols as had the hydroxyl group attached to an aliphatic side chain.[83] In common with the acetylation methods, tertiary alcohols were dehydrated partially, for example:

$$(CH_3)_3COH \xrightarrow{BF_3 + CH_3COOH} (CH_3)_2C{=}CH_2 + H_2O \qquad (18)$$

But, since this reaction liberated the same amount of water per hydroxyl group as would be given by esterification, it was not necessary to isolate these two effects; 1 mole of water was formed per hydroxyl group by either route.

That dehydration occurred with tertiary alcohols was demonstrated in an experiment in which *tert*-butanol was treated with a reagent containing 100 grams of boron trifluoride per liter of methanol. The net water found, after 2 hours at 60°C., was equivalent to 37 per cent dehydration.

Except in the case of phenols, which did not react completely, the method was useful over a wide range of compositions and was remarkably precise in the presence of large amounts of water, acids, or easily hydrolyzed esters.

Another advantage of the method lay in the excellent solvent action of boron fluoride–acetic acid reagent. As the solution remained predominately organic in nature throughout the analysis, materials which were insoluble in an aqueous medium tended to remain dissolved, thereby eliminating the difficulties inherent in a heterogeneous titration. Stearyl thiodipropionate, which was essentially insoluble in water, for example, could be analyzed for traces of free alcohol without difficulty, a typical sample analyzing 0.56 ± 0.01 per cent octadecanol.[84]

[34] Unpublished results from the authors' laboratory.

B. Development of Fischer Reagent Hydroxyl Procedure

It was observed in the authors' laboratory that synthetic products containing acetic acid, methanol, methyl acetate, water, and boron trifluoride underwent marked changes on standing.[84] After a brief period at room temperature, the mixtures initially containing large amounts of free acid with lesser quantities of alcohol and ester were found to contain considerably increased amounts of ester with correspondingly decreased alcohol and acid. The course of the reaction was followed by the changes in water content as determined by the Fischer titration. In one series of experiments, 1 ml. portions of acetic acid, methanol and methyl acetate were mixed with 2 ml. of $BF_3 \cdot 2.5H_2O$ and diluted immediately to 100 ml. with glacial acetic acid and dioxane, respectively. At intervals, 10 ml. aliquots were withdrawn and analyzed for water. No marked change occurred in the dioxane solution. The results for the acetic acid solution are shown in Table XCI.

TABLE XCI

Esterification at Room Temperature of Methanol in Known Solution[84,a]

Standing time	Water found, mg.		Methanol found,[c] %
	Total	Net (corr. for calculated free water)[b]	
5 minutes	156.8	7.0	2.1
	158.1	8.3	2.5
	160.5	10.7	3.2
1 hour	188.8	39.0	11.8
	188.2	38.4	11.6
2 hours	193.2	43.4	13.1
18 hours	195.7	45.9	13.9
	195.2	45.4	13.7

[a] Calculated composition before diluting with acetic acid: 13.3 wt. % methanol, 17.7% acetic acid, 15.7% methyl acetate, 21.3% water, and 32.0 % boron trifluoride.

[b] Calculated free water in 10 ml. aliquot = 149.8 mg.

[c] Calculated from net water formed on basis of original sample.

The data of Table XCI indicated that esterification was complete in about 3 hours at room temperature. (Similar mixtures using ethanol and propionic acid gave essentially complete esterification only after 26 hours at room temperature or 2 hours at 60°C.) The experiments indicated that boron trifluoride dissolved in acetic acid might yield an effective general esterification reagent. A solution was prepared in glacial acetic acid containing about 100 grams of boron trifluoride per liter. Ten and 20 ml. portions of this reagent were added to 5 ml. aliquots containing about 10 millimoles of various alcohols in dioxane. The results of typical experiments are shown in Table XCII.

TABLE XCII

Esterification of Alcohols Using Boron Trifluoride in Acetic Acid (100 grams of boron trifluoride per liter)[84]

Alcohol[a]	Volume of reagent, ml.	Weight per cent of theoretical hydroxyl		
		1 hour at 60°C.	2 hours at 60°C.	2 hours at 67 ± 2°C.
Methanol	20	100	100	100
Ethanol	20	95	100	100
n-Propanol	20		99.5	100
Isobutanol	20		98.5	100
tert-Butanol	20		99.5	100
Isopentanol	20		75	99.5–100
sec-Pentanol	20		75	99.5–100
Cyclohexanol	20		89	100
Methanol	10		100	100
Ethanol	10		100	100

[a] Approximately 10 millimoles of alcohol used for each experiment.

C. Reagents

The esterification catalyst stock solution is $BF_3 \cdot 2CH_3COOH$, equivalent to about 360 grams of boron trifluoride per liter. Approximately 210 ml. (278 grams) of this solution diluted to 1 liter with glacial acetic acid gives the catalyst for the general procedure containing 100 grams of boron trifluoride per liter.

A catalyst solution almost as effective was prepared from the commercially available boron trifluoride etherate ($BF_3 \cdot C_2H_5OC_2H_5$), although a concentration equivalent to about 150 grams per liter was required. About 70 ml. (78.5 grams) of the boron trifluoride catalyst was diluted to 250 ml. with glacial acetic acid. With this reagent and following the conditions of the general procedure, results approximately 99 per cent quantitative were obtained on ethanol and ethylene glycol.

This esterification reagent tended to darken on standing; it was better to prepare and use the solution as needed. Purification of the etherate by vacuum distillation might have given a more stable reagent.

Other reagents include technical dioxane, c.p. glacial acetic acid, and c.p. pyridine.

PREPARATION OF STOCK SOLUTION OF $BF_3 \cdot 2CH_3COOH$

The concentrated esterification catalyst is prepared in the ratio of 1 mole of boron fluoride to 2 moles of acetic acid.[84] This stock solution then may be diluted to the concentration prescribed for the analysis. The necessary apparatus, illustrated in Figure 42, includes a cylinder of boron trifluoride gas connected through a cold trap to a 3 liter, three-neck, round-bottom flask. The trap, kept below —15°C. (ice–methanol), tends to remove any relatively high-boiling impurities in the gas and acts as a safety reservoir in case the liquid

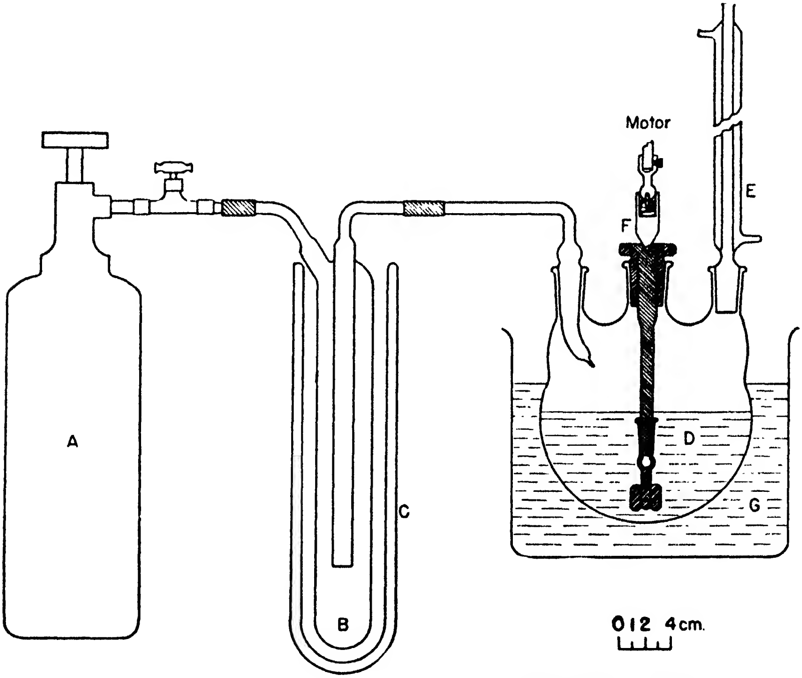


Fig. 42. Apparatus for preparation of $BF_3 \cdot 2CH_3COOH$.[84]

(A) BF_3 cylinder with Hoke needle valve
(B) cold trap
(C) Dewar flask, quart size
(D) flask containing acetic acid
(E) reflux condenser
(F) stirrer, vacuum, Eck & Krebs No. 4550
(G) water–ice bath

is drawn back through the feed line. The inlet tube in the flask is drawn down to about a 1 mm. opening; it should be arranged so that the tip will remain above the liquid level, taking into consideration the total expansion during the absorption of the boron trifluoride. All connections are either ground-glass joints or are glass-to-glass butt joints covered with tightly fitting short pieces of pressure tubing. To prepare about 2 liters of $BF_3{\cdot}2CH_3COOH$, about 1.3 liters of glacial acetic acid are weighed to the nearest gram into the round-bottom flask. Stirrer, condenser, and inlet tube are connected (Fig. 42) and the partially filled water–ice bath is placed around the flask. With the stirrer running, the boron trifluoride cylinder valve is opened slightly and the Hoke valve is adjusted so that the boron trifluoride gas forms a dense white cloud above the acetic acid but does not escape through the condenser into the atmosphere.

The preparation should be carried out in a well-ventilated hood. Boron trifluoride is extremely toxic and, after combination with moisture from the air, is very corrosive. It is best *not* to use a desiccant tube at the exit end of the condenser, since the gas tends to form plugs with the desiccant. The small amount of moisture which may be absorbed without the use of a protecting tube is negligible.

Then the bath is filled immediately with ice to a level above the liquid in the flask. (If the bath is filled with ice initially, the acetic acid may freeze; after reaction with boron trifluoride, however, the melting point is lowered below 0°C.) The flow of gas is continued until about 900 grams have been added (the contents of a 2 lb. cylinder), being careful to keep the bath surrounding the flask well replenished with ice, since the addition reaction is strongly exothermic.

Usually some crystalline $BF_3{\cdot}CH_3COOH$ precipitates if the concentration of boron trifluoride becomes appreciably greater than $1BF_3{:}2CH_3COOH$. On dilution with acetic acid to give the desired 1:2 concentration, a homogeneous solution is obtained.

Actual quantity of boron trifluoride absorbed is determined with sufficient accuracy by further increase in weight of the flask to the nearest gram. Unless an appreciable quantity of boron trifluoride is lost it will be necessary to add about 200 ml. of acetic acid to adjust the solution concentration to the desired $BF_3{\cdot}2CH_3COOH$ (900 grams of boron trifluoride per 1592 grams of acetic acid). This stock solution may be stored safely in rubber-stoppered Pyrex bottles, while catalysts of lower boron trifluoride content are more conveniently handled in glass-stoppered Pyrex bottles.

D. General Analytical Procedure

A portion of sample containing up to 200 milliequivalents of hydroxyl (total hydroxyl plus water should be no more than 300 milliequivalents) is weighed into a 100 ml. glass-stoppered volumetric flask about one third filled with technical dioxane or glacial acetic acid. This mixture is made up approximately to volume with more of the solvent, is shaken until homogeneous, and is finally adjusted to the mark; the solution is thermostated if later samples are to be taken. Five ml. of the sample solution is transferred exactly to a 250 ml. glass-stoppered volumetric flask and 20 ml. of the catalyst solution (100 grams of boron trifluoride per liter) added. The loosely stoppered flask, together with a control containing 20 ml. of catalyst and 5 ml. of solvent, is placed in a water bath at 67 ± 2°C. A water bath of the type shown in Figure 43 is convenient for this purpose.*

Where convenient, the sample, containing up to 10 milliequivalents of hydroxyl plus no more than 5 milliequivalents of water, may be weighed directly into the 250 ml. sample flask. In this case a portion of the original sample is titrated directly for water and no solvent is used in the control.

After momentarily allowing air above the liquid to expand, the flasks are stoppered tightly and left for 2 hours.

An oven may be used in place of the water bath. In this case the flask should be stoppered tightly and sealed with a simple spring clamp (such as item No. 4799 of the Precision Scientific Company, 1730 N. Springfield Ave., Chicago), which serves to prevent expulsion of the stopper on heating.

At the end of this time the flasks are removed and allowed to cool to room temperature spontaneously. Then 5 ml. of C.P. pyridine are added carefully to each flask.

The reaction between pyridine and the acid catalyst is strongly exothermic. If the pyridine is added from a pipet, the tip of which is rotated continuously

---

*The water bath employs two variac-controlled immersion heaters, one fixed and one controlled through the thermoregulator. A centrifugal pump (American Instrument Co., Type 110, 5 amp., 115 volts) is used to maintain a uniform temperature throughout the bath, which when necessary can be controlled to ±0.1°C.

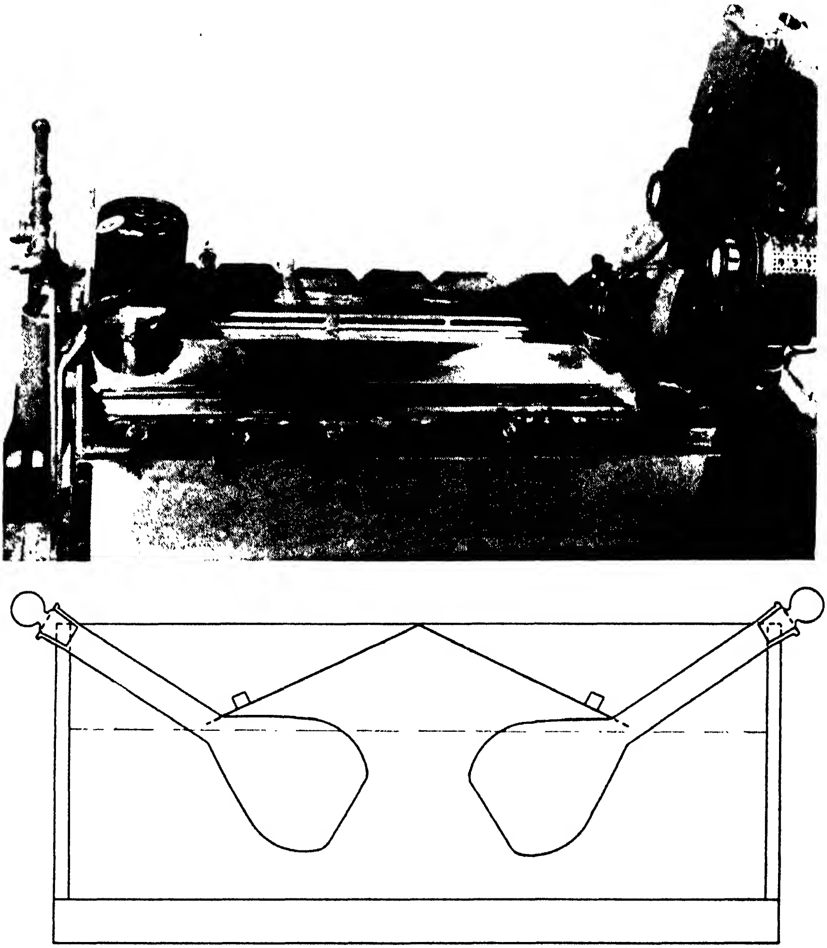


Fig. 43. Constant temperature water bath for volumetric flasks.[84]

around the neck of the flask below the grind, there is no apparent loss of vapor. The addition of pyridine just before titration is essential. Its probable function is to destroy the activity of the catalyst and prevent esterification of the methanol of the Karl Fischer reagent.

In the absence of pyridine, a false endpoint is obtained after the addition of the first few milliliters of Fischer reagent. If the titration is stopped temporarily at this point, the interfering esterification reaction proceeds rapidly, resulting in impossibly high titers. If the titration is continued without pause the false endpoint will be maintained beyond the true endpoint for those samples containing only relatively small quantities of water. These false endpoints with Fischer reagent in the presence of boron trifluoride in acetic acid (or in methanol) are the only cases of this phenomenon observed in this laboratory in which more than traces of water are involved (see page 103, Chapter V).

The mixtures are then titrated with Karl Fischer reagent. The water originally present may be obtained by titrating with Fischer reagent a 5 ml. portion of the original dioxane or acetic acid solution of the sample.

After correction for water in the catalyst, pyridine, solvent, and sample, the net water found is equivalent to the free hydroxyl in the sample.

Most of the correction (water in the catalyst, pyridine, and solvent) is automatically determined by the control. However, the difference in the amount of solvent in the sample and control solutions and the water content of the sample itself must be considered. In calculating the water content of the sample, a correction must be made for the water initially present in the solvent. This correction is based on the volume of solvent taken in the aliquot and is figured with sufficient precision as the difference between the total volume of aliquot and the volume of concentrated sample analyzed, neglecting volume changes on mixing.

The following is an example of a typical analysis with the required calculations: 7.9163 grams (10 ml.) of isobutanol (M.W. = 74.1) were diluted to 100 ml. with dioxane. A 5 ml. portion contained 0.3958 gram (0.5 ml.) of alcohol plus 4.5 ml. of dioxane.

Solution	Fischer reagent, ml. (0.1653 millimole H_2O/ml.)	Water found, millimoles	
		Gross	Net
5 ml. dioxane	1.5, 1.5	(*a*) 0.25	(*a'*) 0.22/4.5 ml. dioxane
5 ml. sample aliquot	2.5, 2.4	(*b*) 0.40	(*b'*) $0.18 = (b - a') \approx 0.8\% \, H_2O$
5 ml. dioxane + 20 ml. catalyst (control)	22.4, 22.4	(*c*) 3.70	(*c'*) $3.67 = (c - a + a')$
5 ml. sample + 20 ml. catalyst	55.2, 55.3	(*d*) 9.13	(*d'*) $5.28 = [d - (c' + b')]$

$$\frac{5.28 \times 74.1}{1000 \times 0.3958} \times 100 = 98.9\% \text{ isobutanol}$$

$$98.9\% + 0.8\% = 99.7\% \text{ total}$$

E. Analytical Results

1. REACTION OF ALIPHATIC AND ALICYCLIC ALCOHOLS

The experimental results obtained on a number of alcohols are given in Table XCIII. In most cases the substances were analyzed as received without further purification.

TABLE XCIII

Analytical Data for Alcohols[83,84]

Substance	Hydroxyl compound,[a] %	Complete analysis using Karl Fischer reagent		
		Hydroxyl compound,[b] %	Water, %	Total, %
Methanol		(6) 99.0 ± 0.4	0.5	99.5
Ethanol		(4) 99.5 ± 0.2	0.4	99.9
Ethylene glycol		(8) 99.8 ± 0.3	0.2	100.0
n-Propanol		(2) 98.9 ± 0.4	1.2	100.1
Allyl alcohol	97.2	(4) 96.6 ± 0.3	1.9	98.5
Glycerol		(2) 94.3 ± 0.3	4.8	99.1
Isobutanol		(6) 98.8 ± 0.2	0.9	99.7
tert-Butanol		(6) 99.2 ± 0.3	0.8	100.0
Butynediol		(4) 99.2 ± 0.3	0.7	99.9
Pentaerythritol		(6) 99.5 ± 0.2	0.4	99.9
2-Methyl-4-butanol	97.8	(6) 97.3 ± 0.4	1.0	98.3
2-Pentanol		(4) 98.3 ± 0.5	1.6	99.9
2-Methyl-2-butanol		(4) 99.3 ± 0.2	0.9	100.2
2,4-Dimethyl-3-pentanol		(2) 99.8 ± 0.3	0.2	100.0
Citronellol[c]	91.3	(3) 91.7 ± 0.1	0.7	92.4
Geraniol[c]	70.0	(6) 109.0 ± 0.3	0.1	109.1
Linaloöl		(2) 99.3 ± 0.1	0.7	100.0
Dodecanol[d]	98.2	(2) 98.5 ± 0.3	0.0	98.5
Tetradecanol[d]	92.2	(2) 91.8 ± 0.0	0.0	91.8
Hexadecanol[d]	88.8	(4) 89.0 ± 0.2	0.0	89.0
Octadecanol[d]	96.2	(2) 96.3 ± 0.3	0.0	96.3
Cyclohexanol	97.3	(2) 98.1 ± 0.2	0.1	98.2
Borneol	98.4	(4) 98.4 ± 0.2	0.0	98.4
Menthol	99.5	(4) 98.5 ± 0.2	0.0	98.5
Terpin hydrate		(2) 90.4 ± 0.4	9.5	99.9
α-Terpineol[c]		(4) 105.0 ± 0.2	1.3	106.3
Fenchyl alcohol[c]	86.5	(2) 87.1 ± 0.3	1.3	88.4
Santalol[c]	89.4	(3) 95.8 ± 0.2	0.4	96.2
Benzyl alcohol	97.1	(4) 97.3 ± 0.2	1.1	98.4

[a] Acetyl chloride–pyridine procedure.[32]
[b] Figures in parentheses represent number of individual determinations.
[c] Technical grade chemical, all others C.P.
[d] Cut from fractionation of crude alcohol mixture.

The precision and accuracy averaged about ±0.3 per cent, comparing very favorably with the involved acetic anhydride procedures. The essentially stoichiometric accuracy obtained in most cases appeared to be somewhat better than that of the acetyl chloride–pyridine method which usually approached 99 per cent rather than 100 per cent.[32]

The results obtained on the terpene alcohols were interesting since they involved primary, secondary, and tertiary hydroxyl groups as well as active unsaturated linkages. Of the three olefinic terpene alcohols studied, only geraniol gave results above theoretical, while in the monocylic series only α-terpineol gave high values. The lower value for geraniol by the acetyl chloride procedure suggested that the geraniol and α-terpineol may have contained appreciable concentrations of impurities with tertiary hydroxyl groups. Usually, α-terpineol was prepared by dehydration of the dihydric compound, terpin hydrate; as little as 5 per cent of the latter in technical terpineol would explain the high results for the former. The 2 bicyclic terpenes, borneol and fenchyl alcohol, gave values agreeing fairly well with those found by the acetyl chloride procedure. The bicyclic olefinic alcohol, santalol, gave values somewhat higher than that given by the acetylation method, possibly due to tertiary hydroxyl. Menthol, on the other hand, consistently gave slightly lower results by the procedure using Fischer reagent; use of a catalyst containing 200 (instead of 100) grams of boron trifluoride per liter of acetic acid solution gave a value of 99.8 ± 0.2 per cent for this compound.[84]

TABLE XCIV

Analytical Data for Aqueous Alcohol Solutions[83]

Alcohol		Weight per cent by analysis		
Name	Weight per cent by difference	Alcohol	Water	Total
Ethanol	0.8	1.0 ± 0.2	99.2 ± 0.0	100.2
Ethanol	3.9	3.9 ± 0.2	96.1 ± 0.1	100.0
Ethanol	7.6	7.4 ± 0.3	92.4 ± 0.0	99.8
Ethanol	15.8	15.6 ± 0.2	84.2 ± 0.0	99.8
Ethanol	19.4	19.0 ± 0.2	80.6 ± 0.2	99.6
Ethanol	38.9	39.0 ± 0.3	61.1 ± 0.1	100.1
n-Propanol	17.8	17.9 ± 0.2	82.2 ± 0.1	100.1
Isobutanol	8.3	8.3 ± 0.2	91.7 ± 0.0	100.0
tert-Butanol	16.2	16.0 ± 0.2	83.8 ± 0.1	99.8
n-Pentanol	3.5	3.8 ± 0.2	96.5 ± 0.1	100.3
Isopentanol	2.2	2.5 ± 0.2	97.8 ± 0.0	100.3

The general method also was useful in the determination of alcohols containing large quantities of water. In Table XCIV are collected results obtained on several alcohols containing up to about 99 per cent water. In each case the alcohol added was calculated as the difference between the per cent water found by analysis and 100 per cent.

2. Reaction of Phenols

Phenols did not esterify completely under the conditions of the general procedure even with increased concentrations of boron trifluoride in the acetic acid reagent. This was evident from the data reported in Table XCV, for which approximately 10 milliequivalent portions of phenolic hydroxyl compounds were treated with 20 ml. portions of esterification reagents (having various strengths of boron trifluoride) and heated for 2 hours at 67 ± 2°C. No advantage was gained by increasing the heating period to 4 hours or by substituting trimethylacetic, dichloroacetic, trifluoroacetic, or *n*-caproic acids for acetic acid in the catalyst solution. By using acetic anhydride* in place of acetic acid, roughly quantitative results were obtained; however, the endpoints were so obscure, because of unusual darkening of the solution, that analyses were precise and accurate to only about ±4 per cent. (Electrometric endpoints were not investigated.)

TABLE XCV

Esterification of Phenols with Boron Trifluoride–Acetic Acid Reagent[84]

Substance	Per cent of theoretical hydroxyl using acetic acid containing			
	100 g. BF_3/l	200 g. BF_3/l.	300 g. BF_3/l.	360 g. BF_3/l. ($BF_3 \cdot 2CH_3COOH$)
Phenol	75.5	86.5	93.5	100.0
Resorcinol		55.0		85.0
Catechol	50.0			87.5
Pyrogallol	67.0			70.0
Phloroglucinol	58.0	60.5		63.0
α-Naphthol	65.5		76.5	

3. Determination of Aliphatic Alcohols in the Presence of Phenols

The differences in the relative esterification rates of aliphatic alcohols and phenols made possible methods for differentiating these two classes of compounds in selected cases. Thus, the concentration

---

* With appropriate procedure modifications, involving hydrolysis of the excess anhydride with a known quantity of water (see Chapter XI).

of boron trifluoride catalyst and the reaction temperature could be reduced to a point where the phenols reacted only slightly while the esterification of certain aliphatic alcohols remained essentially complete. This selective action probably was due as much to differences in the equilibrium constants of the two series as to purely kinetic differences. Experimental results from the use of less concentrated catalyst solutions are given in Table XCVI.

TABLE XCVI

Analytical Results with Alcohols Using Less Concentrated Catalysts in Acetic Acid Solutions[83]

Substance	Per cent of theoretical hydroxyl			
	2 hours at 26 ± 1°C.		2 hours at 60°C.	
	25 g. BF$_3$/l.	50 g. BF$_3$/l.	25 g. BF$_3$/l.	50 g. BF$_3$/l.
Ethanol	25	45	96.8 ± 0.3	99.0 ± 0.0
n-Propanol			98.7 ± 0.3	
Isobutanol			96.7 ± 0.3	
tert-Butanol	83	93	95.5 ± 0.4	99.1 ± 0.4
Ethylene glycol			90.6 ± 0.2	
Glycerol			78.1 ± 0.2	
Cyclohexanol			55.9 ± 0.0	
Benzyl alcohol			97.2 ± 0.4	
Phenol	0.0	0.0	3.9 ± 0.2	26.6 ± 0.2
Guaiacol	0.0	0.0	4.5 ± 0.2	28.3 ± 0.5

The high activity of *tert*-butanol at room temperature was due primarily to dehydration since esterification to this extent seemed unlikely. The general procedure was altered slightly to permit the estimation of certain aliphatic in the presence of aromatic alcohols; those changes involved employing a reagent containing 25 grams of boron trifluoride per liter of acetic acid solution and heating for 2 hours at 60°C. Using this modified procedure, a few ethanol–phenol mixtures were analyzed, with the results summarized in Table XCVII.

TABLE XCVII

Analytical Results on Determination of Ethanol in Presence of Phenol[83]

Phenol added, %	Ethanol added, %	Ethanol found,[a] %
93.3	6.7	9.0 ± 0.2
57.0	43.0	43.8 ± 0.2
11.1	88.9	88.2 ± 0.1

[a] No attempt was made to apply factors based on Table XCVI.

The results obtained on phenol and guaiacol (Table XCVI) probably were typical of what might be expected with other phenols since the latter reacted to no greater extent than phenol when more concentrated catalyst solutions were used (Table XCV). Also the degree of differentiation between other aliphatic and aromatic alcohols would probably be much like that predicted by Table XCVI.

F. Interfering Substances

It already has been shown that unsaturates and water in general did not interfere in the determination of alcoholic hydroxyl groups using Fischer reagent. Ethers tended to be inert. No marked effect on the accuracy was noticed in the presence of high concentrations of acids. The behavior toward the general procedure of alcoholic hydroxyl compounds containing acid, ester, and ether groups is illustrated in Table XCVIII.

TABLE XCVIII

Analytical Data for Hydroxy Acids, Esters, and Ethers[83,84]

Substance	Hydroxyl compound (various methods), %	Complete analysis using Karl Fischer reagent: Hydroxyl compound,[a] %	Water, %	Total, %
Glycolic acid	99.9[b]	(4) 100.0 ± 0.3	0.0	100.0
α-Hydroxyisobutyric acid	97.6[b]	(2) 97.5 ± 0.3	0.1	97.6
Ricinoleic acid[c]	58.3[b]	(2) 58.9 ± 0.0	1.7	60.6
Tartaric acid	94.5[b]	(2) 94.1 ± 0.4	0.1	94.2
Methyl glycolate		(2) 99.7 ± 0.2	0.1	99.8
Methyl lactate[c]	97.4[d]	(2) 97.5 ± 0.0	0.4	97.9
Ethyl lactate	98.7[d]	(2) 98.5 ± 0.3	0.4	98.9
Ethyl tartrate	94.7[d]	(2) 93.9 ± 0.0	0.0	93.9
Glycerol β-methyl ether		(2) 99.0 ± 0.5	0.5	99.5
Benzoin[e]	93.2[f]	(4) 97.4 ± 0.2	0.0	97.4
Methyl Cellosolve	93.2[f]	(2) 94.5 ± 0.2	4.5	99.0
Cellosolve	100.6[f]	(2) 101.0 ± 0.1	0.3	101.3
Methyl carbitol[c,g]	130.0[f]	(2) 129.7 ± 0.4	0.4	130.4
Glycol ricinoleate	(No.) 222[f]	(2) (No.) 221 ± 1.0	0.1	
Glucono-δ-lactone	103.1[f]	(4) 103.3 ± 0.3	0.2	103.5

[a] Figures in parentheses represent number of individual determinations.
[b] By alkali titration.
[c] Technical grade, all others C.P.
[d] Saponification.
[e] Benzoin, which contains a carbonyl group, is included in this table for convenience; see Table XCIX.
[f] Acetyl chloride procedure.
[g] Contained free glycol.

The tertiary hydroxyl groups of citric acid, ethyl citrate, and benzilic acid were esterified to the extent of 35, 44, and 47 per cent, respectively. (Based on titration of the benzilic acid with standard sodium methylate in methanol, the purity was 99.4 per cent.)

A sample containing 98.8 per cent methyl formate analyzed 1.1 ± 0.0 per cent methanol and 0.05 per cent water, while an 89.6 per cent solution of methyl acetate gave 0.7 ± 0.1 per cent methanol and 9.8 per cent water. A sample containing 95 per cent acetic acid analyzed 3.9 ± 0.1 per cent ethanol and 1.0 per cent water. Esters in general (except ortho esters but including other easily hydrolyzed compounds) did not interfere.

An interesting application of Fischer reagent hydroxyl procedure concerned the analysis of ethylene glycol formate mixtures, containing formic acid, water, free glycol, and mono-, and diformate. The net hydroxyl value between the total found by this method and free glycol by periodate oxidation[72-74] was a measure of ethylene glycol monoformate. For example, one sample gave a total hydroxyl number of 650 and a periodate hydroxyl number of 307. The net value of 343 was equivalent to 55.0 per cent monoformate. The complete analysis of this sample was:

Substance	Weight per cent found	Method
Formic acid	4.7	Titration with sodium methylate solution
Ethylene glycol	17.0	Direct periodate oxidation
Water	2.5	Direct Fischer titration
Ethylene glycol monoformate	55.0	Karl Fischer hydroxyl method (corrected for free glycol)
Ethylene glycol diformate	21.0 (aver.)	21.5% based on net saponification value (after correction for free acid + monoformate) 20.6% based on net periodate value after saponification (after correction for free glycol + glycol from monoformate)

Behavior of diethyl peroxide was interesting. A solution of the peroxide,* showing a strong hydroxyl band by infrared analysis, gave

---

* This sample analyzed 77 per cent peroxide (see Chapter XIV for method of analysis) and was known to contain diethyl ether.

values of 10.8 ± 0.2 per cent ethanol and 1.2 per cent water. After sufficient ethanol was added to give a total calculated content of 21.0 per cent alcohol, the solution analyzed 21.3 ± 0.0 per cent ethanol.

The general hydroxyl procedure was unfavorably affected by the presence of a few classes of organic compounds. Aldehydes reacted to a considerable extent, although ketones, with the exception of cyclohexanone, reacted only slightly. The reaction of carbonyl compounds in general was probably analogous to that proposed for formaldehyde:[83]

$$CH_2O + 2\,CH_3COOH \longrightarrow (CH_3COO)_2CH_2 + H_2O \tag{19}$$

The extent of reaction of a few aldehydes and ketones, after 2 hours at 67 ± 2°C., with acetic acid containing various quantities of boron trifluoride is shown in Table XCIX.

TABLE XCIX

Reaction of Carbonyl Compounds with Acetic Acid in Presence of Boron Trifluoride[83,84]

Substance	Per cent reaction using acetic acid containing				
	25 g. BF_3/l.	50 g. BF_3/l.	100 g. BF_3/l.	200 g. BF_3/l.	300 g. BF_3/l.
Formaldehyde[a]	4	20	70	73	72
n-Butyraldehyde	10–20		60	70	
2-Ethyl-1-hexanal	10	36	42.5		66
Acetone	0.0	0.0	2.0	13	
Methyl ethyl ketone		0.0	0.0	10	
Ethyl isopropyl ketone	0.0	0.0	0.0		7.5
Cyclohexanone	10	18	48		60

[a] 37% aqueous, all others C.P. grade.

The data, indicating that the interference of aliphatic ketones was negligible if no more than 100 grams per liter of boron trifluoride catalyst was used, were supported by an analysis of a 58 per cent isopropanol–42 per cent acetone mixture for which 58.5 ± 0.0 per cent of the alcohol was found.[84] Benzoin (Table XCVIII) was esterified with no more than minor interference from the carbonyl group. A 50–50 cyclohexanol–cyclohexanone mixture, however, analyzed 73 per cent as cyclohexanol.[84]

Acetals and ketals reacted nearly to completion, assuming 2 moles of water were formed for each mole of compound. For methylal the reaction could be written:[83]

$$CH_2(OCH_3)_2 + 4\ CH_3COOH \longrightarrow 2\ CH_3COOCH_3 + (CH_3COO)_2CH_2 + 2\ H_2O \qquad (20)$$

Several acetals were treated with boron trifluoride in acetic acid reagents and heated for 2 hours at 67 ± 2°C. Results are shown in Table C.

TABLE C

Reaction of Acetals with Acetic Acid in Presence of Boron Trifluoride[83,84]

Substance	Per cent reaction using acetic acid containing		
	25 g. BF_3/l.	100 g. BF_3/l.	200 g. BF_3/l.
Methylal	56.0	92.0	
Acetal	53.5	91.5	
Ethylal	50.5	78.0	85.0
n-Propylal	46.0	77.5	85.0
Methoxymethoxyethanol[a]	13.0	50.0	115.0
Di-*n*-butylmethylal		88.5	
Dimethoxymethylal[b]		90.0	105.0
Dibutoxymethane		80.0	83.5
1,3-Dioxolane		85.0	
Dichloroethylmethylal		80.5	88.0

[a] $CH_3OCH_2OCH_2CH_2OH$; results calculated on basis of 1 mole of water per mole of compound, after correction for quantitative reaction of the hydroxyl group.

[b] $H_2C(OCH_2OCH_3)_2$.

Ortho esters, as contrasted to normal esters, apparently reacted quantitatively with the acetic acid reagent, probably according to the reaction:[85]

$$RC(OR')_3 + 2\ CH_3COOH \longrightarrow RCOOR' + 2\ CH_3COOR' + H_2O \qquad (21)$$

Five "C.P." ortho esters were mixed with the 100 grams per liter of boron trifluoride–in–acetic acid catalyst and heated for 2 hours at either 60° or 67°C. The same values were obtained at both temperatures. Analytical results are summarized in Table CI.

Mercaptans were esterified by the acetic acid–boron trifluoride reagent, according to the equation:[84]

$$RSH + CH_3COOH \longrightarrow CH_3COSR + H_2O \qquad (22)$$

Approximately 8 millimole quantities of the mercaptans in acetic

---

[85] Smith, D. M., Mitchell, J., Jr., and Hawkins, W., *J. Am. Chem. Soc.*, **66**, 715–6 (1944).

TABLE CI

Analytical Data for Ortho Esters[85]

Substance	Found, wt. %
Ethyl orthoformate	97.6 ± 0.4
Methyl orthoacetate	99.4 ± 0.3
Methyl orthopropionate	97.4 ± 0.0
Methyl ortho-*n*-butyrate	97.1 ± 0.0
Methyl ortho-*n*-valerate	92.2 ± 0.3

acid solution were analyzed according to the general hydroxyl procedure (100 grams of boron trifluoride per liter, 2 hours at 67°C.). Typical results, together with comparative data by the iodine oxidation method,[86] are given in Table CII. Like the phenols, thiophenol reacted only partially giving a value of 70 per cent compared to 95.8 per cent by the iodimetric method.[84]

TABLE CII

Analytical Data for Mercaptans[84]

Substance	Mercaptan found, wt. %	
	Esterification	Iodine oxidation
Isopropyl mercaptan	91.8 ± 0.2	92.2
n-Butyl mercaptan	96.2 ± 0.3	97.9
Heptyl mercaptan	94.5 ± 0.1	94.2
Benzyl mercaptan	97.2 ± 0.2	98.4
Thioglycolic acid	92.5 ± 0.3	93.6

Nitriles interfered through addition of water—extent of reaction varying with the boron trifluoride concentration.[84] Samples of acetonitrile were diluted with dioxane and acetic acid, respectively. Five milliliter portions, containing 5 millimoles of nitrile, were treated with 20 ml. of a 100 grams of boron trifluoride per liter catalyst solution containing 15 millimoles of water. (Normally 2–3 millimoles of water were present for anhydrous samples plus standard reagents.) After 1 hour at room temperature or at 60°C., the reactions of the dioxane aliquots were negligible, but after 2 hours at 65°C., water had been absorbed equivalent to 4.6 per cent nitrile on an equimolar basis. For the acid aliquots the corresponding results were 1, 3.5, and 9 per cent. A sample of formaldehyde cyanohydrin analyzed 95 per cent pure by the general Fischer reagent

---

[86] Kimball, J. W., Kramer, R. L., and Reid, E. E., *Ibid.*, 43, 1199–1200 (1921).

hydroxyl method, as compared with a value of 99.5 per cent by the acetyl chloride procedure.[32] On the other hand, acetaldehyde cyanohydrin analyzed 40 per cent pure, as compared to 99 per cent by the acetyl chloride procedure.[87]

No interference was observed with amides or anilides. Results of 0.0 per cent were obtained on acetamide, adipamide, acetanilide, and propionanilide.

Most amines interfered by affecting catalyst activity. However, the esterification reaction could be made quantitative by limiting the quantity of amine to no more than 5 millimoles or by increasing the catalyst concentration, as demonstrated in section H of this chapter.

G. Other Studies

Comparative esterification data were obtained at room temperature on 5 ml. aliquots of methanol in dioxane solution (4.0 grams diluted to 100 ml.), using 20 ml. portions, respectively, of 1.47 *M* boron trifluoride catalyst (100 g. BF_3/l.), 1.47 *M* sulfuric acid, and 1.47 *M* phosphoric acid, all in acetic acid:

Reagent	Hours at 26 ± 2°C.	Methanol found, wt. %
BF_3	2	73.1 ± 0.0
	3	91.5 ± 0.2
	4	99.8 ± 0.0
H_2SO_4	2	70 ± 1.0
	3	90 ± 2.0
	4	94 ± 1.0
H_3PO_4	2	33
	3	43
	4	51

Dichloroacetic acid was also studied as a selective esterification reagent:[84]

$$ROH + Cl_2CHCOOH \longrightarrow Cl_2CHCOOR + H_2O \qquad (23)$$

This strong organic acid was used directly without additional catalyst, under the otherwise identical conditions for the general method.

A sample containing about 10 milliequivalents of hydroxyl is treated with 20 ml. of dichloroacetic acid containing about 0.3 per

---

[87] Mitchell, J., Jr., and Hawkins, W., *Ibid.*, 67, 777–8 (1945).

cent water,* and heated for 2 hours at 67 ± 2°C. The solution then is allowed to cool and 30 ml. of a 1 : 1 pyridine:dioxane solution are added. (Pyridine is added to combine with the free acid, and dioxane is added to act as a solvent for the pyridine-dichloroacetic acid salt thus formed.) The mixture is titrated directly with Karl Fischer reagent.

Analyses were made on concentrated samples weighed directly into the 250 ml. volumetric flasks and also on 5 ml. aliquots of samples in 100 ml. of dioxane or acetic acid solution. Comparative results are given in Table CIII.

TABLE CIII

Analytical Results on Determination of Alcohols Using Dichloroacetic Acid[84]

Substance	Per cent of theoretical hydroxyl		
	Direct	Dioxane solution	Acetic acid solution
Ethanol	96.0 ± 0.1	93.3 ± 0.2	95.5 ± 0.2
n-Propanol	97.9 ± 0.2	91.0 ± 0.0	96.6 ± 0.0
n-Butanol	98.8 ± 0.3		97.6 ± 0.2
Isobutanol	96.9 ± 0.2	92.7 ± 0.2	95.4 ± 0.0
tert-Butanol	94.4 ± 0.0	78.3 ± 0.1	88.1 ± 0.1
Ethylene glycol	68.5 ± 0.5		67.6 ± 0.4
Glycerol	43.0 ± 0.5		51.2 ± 0.5
Cyclohexanol	72.4 ± 0.3	64.9 ± 0.3	71.2 ± 0.2
Benzyl alcohol	79.2 ± 0.2		78.4 ± 0.2
Phenol	0.5 ± 0.1		0.0 ± 0.0
Resorcinol	0.2 ± 0.0		0.3 ± 0.1
Guaiacol	0.2 ± 0.1		0.0 ± 0.0

The precision of the method employing dichloroacetic acid as esterification catalyst was about the same as that of the general method but the accuracy was somewhat lower than that employing the 25 gram boron trifluoride–in–acetic acid reagent with the exception of the butanols and cyclohexanol (Table XCVI). On the other hand, the reaction of phenols was negligible, so that, in an analysis for aliphatic in the presence of aromatic alcohols, an empirical correction could be applied with more assurance. This is illustrated in Table CIV summarizing analyses of known ethanol–phenol mixtures aliquoted from acetic acid.

---

* Commercial dichloroacetic acid contains appreciable quantities of water. The concentration can be reduced readily to about 0.3 weight per cent by fractionation, the water coming off in the distillate.

TABLE CIV

Analytical Results on Estimation of Ethanol in Presence of Phenol Using Dichloroacetic Acid[84]

Phenol, added, %	Ethanol added, %	Ethanol found, %	Ethanol corrected,[a] %
84.6	15.4	14.8 ± 0.3	15.5
54.3	45.7	44.5 ± 0.1	46.5
11.0	89.0	84.0 ± 0.1	88.0

[a] Corrected on basis of 95.5 per cent reaction for ethanol.

The boron trifluoride catalyst method (Table XCVII) had the advantage of compensating errors so that mixtures high in ethanol appeared to have reacted more nearly quantitatively. Mixtures of low ethanol concentration, however, gave better results with dichloroacetic acid.

The interferences using dichloroacetic acid in general were similar to those for the boron trifluoride catalyst procedure. Cellosolve and methyl carbitol analyzed 85.2 and 101.0 per cent, respectively, compared to averages of 101.0 and 129.7 by the procedures of Table XCVIII.

Carbonyl compounds reacted only slightly; results of 7.0, 0.0, and 2.9 per cent were obtained for *n*-butyraldehyde, acetone, and cyclohexanone, respectively. Corresponding values by the 25 grams of boron trifluoride per liter procedure were 10–20, 0, and 10 per cent (Table XCIX).

The dichloroacetic acid method occasionally might be useful for the determination of alcohols in the presence of aldehydes. For example, two solutions containing 51.1 per cent *n*-butanol–48.9 per cent *n*-butyraldehyde and 75.7 per cent *n*-butanol–24.3 per cent *n*-butyraldehyde analyzed 53.3 and 75.9 per cent alcohol, respectively.

Methoxymethoxyethanol gave values averaging 1.7 per cent, after correction for the assumed quantitative reaction of the alcohol. Based on the reactions of other alcohols (Table CIII), esterification of the hydroxyl group of methoxymethoxyethanol would be about 95–96 per cent quantitative. Consequently, the interfering reaction probably was several per cent higher than indicated above.

Methylal and propylal analyzed 45.5 and 35 per cent, respectively, about half the value obtained by using the general procedure (Table C).

The ortho esters appeared to react almost quantitatively. Ethyl orthoformate analyzed 97.6 per cent and methyl-ortho-*n*-valerate, 88.6 per cent. Corresponding values with the boron trifluoride procedure were 97.6 and 92.2 per cent, respectively (Table CI).

H. Determination of Aliphatic Amino Alcohols

The primary and secondary amino groups usually reacted to a variable extent with acetylating agents in a manner similar to that of alcohols. For example, the primary amines reacted according to the equations:

$$RNH_2 + (CH_3CO)_2O \longrightarrow RN(H)OCCH_3 + CH_3COOH \qquad (24)$$

$$RNH_2 + CH_3COCl \longrightarrow RN(H)OCCH_3 + HCl \qquad (25)$$

In some cases, these reactions could be made to go nearly to completion. Stodola[25] indicated that amines reacted almost quantitatively by his micro procedure employing acetic anhydride. Olson and Feldman[88] obtained up to 90 per cent acetylation with acetyl chloride, although aliphatic amines in general gave considerably lower values.

The periodate[89-91] and lead tetraacetate[92] methods have found some application in the analysis of α-alkanol amines. Another method of limited application involved extraction and isolation of the amino alcohol as the hydrochloride salt, liberation of the free amine with silver oxide, and subsequent titration with standard acid.[93] This technique determined basic groups and was not specific for hydroxy amines. Mono- and diethanolamines have been analyzed by precipitation of the *p*-bromobenzene sulfonyl derivatives[94] and triethanolamine, as the hydrochloride, after the mono- and diethanolamine salts had been extracted with isopropanol.[95]

By doubling the standard catalyst concentration in the Karl Fischer esterification procedure, Smith, Mitchell, and Hawkins[85] were able to determine specifically and quantitatively the hydroxyl group in amino alcohols or in the presence of ammonia and amines. The same general reaction (equation 17) was involved where 1 mole

---

[88] Olson, V. R., and Feldman, H. B., *J. Am. Chem. Soc.*, **59**, 2003–5 (1937).
[89] Nicolet, B. H., and Shinn, L. A., *Ibid.*, **61**, 1615 (1939).
[90] Jones, J. H., *J. Assoc. Official Agr. Chemists*, **27**, 462–7 (1944).
[91] Burmaster, C. F., *J. Biol. Chem.*, **165**, 1–6 (1946).
[92] Leonard, N. J., and Rebenstorf, M. A., *J. Am. Chem. Soc.*, **67**, 49–51 (1945).
[93] Jones, J. H., *J. Assoc. Official Agr. Chemists*, **27**, 309–17 (1944).
[94] Shupe, I. S., *Ibid.*, **26**, 754–7 (1941).
[95] Eastland, C. J., Evers, N., and West, T. F., *Analyst*, **62**, 261–6 (1937).

of water was liberated for each equivalent of hydroxyl esterified. Since the reaction of the amine group with the acid or boron trifluoride did not involve water, no interference was encountered.

The procedure was applicable to aliphatic, mono-, and polyhydric amino alcohols in general and to such aromatic compounds as had the hydroxyl group attached to an aliphatic side chain. Amino phenols did not react completely.

1. Development of the Method

20 ml. of various concentrations of boron trifluoride in acetic acid were added to 10 milliequivalent samples of carefully purified mono- and diethanolamine. The mixtures were heated for 2 hours at 65°C., according to the general hydroxyl procedure:

	Per cent of theoretical hydroxyl		
	50 g. BF_3/liter	100 g. BF_3/liter	200 g. BF_3/liter
Monoethanolamine	38	96	99.8
Diethanolamine	79	96	99.9

The higher concentration of catalyst required to effect complete esterification in the presence of this type of compound suggested complex formation between the boron trifluoride and the amine, thereby reducing the effectiveness of the former. A reagent containing 100 grams of boron trifluoride per liter (i.e., the general hydroxyl catalyst) quantitatively esterified the hydroxyl group if only 5 milliequivalents of amine were present. An experiment in which 10 milliequivalents of *n*-butanol and 20 milliequivalents of *n*-butylamine were analyzed using 200 grams of BF_3 per liter catalyst gave only 98.3 per cent recovery of the alcohol. Obviously the ratio of amine to boron trifluoride was a critical factor in the analysis. It was apparent, however, that the catalyst concentration could be adjusted to permit quantitative reaction.

2. Preparation of Reagent

The catalyst solution, containing approximately 200 grams of boron trifluoride per liter of glacial acetic acid solution, is conveniently prepared by diluting approximately 420 ml. (556 grams) of

$BF_3 \cdot 2CH_3COOH$ (see page 275 for preparation) to 1 liter with glacial acetic acid.

3. ANALYTICAL PROCEDURE

The sample containing up to 0.1 equivalent of hydroxyl is weighed into a 50 ml. glass-stoppered volumetric flask about one third filled with acetic acid. (It is usually desirable to chill the flask containing the acetic acid before addition of the sample.) The solution is thermostated and then made up to volume with more acetic acid. Exactly 5 ml. of the sample solution is transferred to a 250 ml. glass-stoppered volumetric flask. (Alternatively the sample containing up to 10 milliequivalents of hydroxyl may be weighed directly into the 250 ml. flask; in this case 5 ml. of glacial acetic acid should be added to the cooled flask containing the weighed sample.) After the addition of 20 ml. of the catalyst solution, the flask is stoppered and, together with a control containing 20 ml. of reagent and 5 ml. of acetic acid, is placed in a water bath or oven at 67 ± 2°C. for 2 hours. At the end of this time the flasks are removed and allowed to cool to room temperature spontaneously. Ten milliliters of dry C.P. pyridine are added carefully to each flask and the mixtures are titrated with Karl Fischer reagent.

After correction for the water in acetic acid, esterification reagent, and pyridine, the millimoles of water present after reaction less the water originally present in the sample are equal to the milliequivalents of free hydroxyl in the sample.

The water originally present may be obtained by titrating a portion of the sample in acetic acid of known water content. If an aliquot of thermostated acetic acid solution is titrated, the adjustment for water present in the acid is obtained by titration of an equal volume of acetic acid alone and correction to the volume of acid actually present in the sample solution (see page 279 for sample calculation).

4. EXPERIMENTAL RESULTS

Analyses of several amino alcohols are summarized in Table CV. With the exception of the carefully purified mono- and diethanol amines, the suppliers' products were used without further purification. The precision and accuracy average about ±0.3 per cent, comparing favorably with the general hydroxyl procedure.

TABLE CV

Analytical Data for Amino Alcohols[85]

Substance	Found, wt. %		
	Amino alcohol[a]	Water	Total
Monoethanolamine	(6) 99.8 ± 0.1	0.1	99.9
Diethanolamine	(4) 99.9 ± 0.1	0.0	99.9
Triethanolamine	(4) 99.9 ± 0.1	0.0	99.9
Diisopropanolamine	(2) 100.1 ± 0.1	0.7	100.8
Triisopropanolamine	(2) 95.0 ± 0.4	0.2	95.2
2-Amino-2-methyl-1-propanol	(4) 99.6 ± 0.2	0.3	99.9
Hydroxyethylethylenediamine	(2) 97.8 ± 0.3	0.6	98.4
2-Amino-2-methyl-1,3-propanediol	(4) 99.5 ± 0.4	0.0	99.5
2-Amino-2-ethyl-1,3-propanediol	(4) 89.9 ± 0.5	0.4	90.3
2-Amino-1-butanol	(4) 96.5 ± 0.3	1.6	98.1
Tris(hydroxymethyl)aminomethane	(4) 99.2 ± 0.2	0.4	99.6
Ethylphenylethanolamine	(2) 96.7 ± 0.2	0.4	97.1

[a] Figures in parentheses represent number of individual determinations.

The results of Table CV demonstrated the general applicability of the procedure to aliphatic amino alcohols containing primary, secondary, and tertiary amine and hydroxyl groups. Those materials analyzing less than 100 per cent probably contained nonhydroxylated impurities. Amino phenols, like phenols in the general method, reacted only partially; the reaction of *m*-aminophenol was only 40 per cent complete.

The types of interference by other functional groups as discussed under the general procedure for alcoholic hydroxyl (page 284 ff.) also would apply to this technique.

CHAPTER X

Determination of Organic Carboxylic Acids

A. Methods of Analysis for Organic Acids

The most commonly employed and most rapid procedures for the determination of acids are based on neutralization with strong alkalies, usually sodium hydroxide. For nonaqueous mixtures containing easily hydrolyzed esters or aldehydes, sodium methylate is a better reagent; in the almost anhydrous environment, interference involving hydrolysis or condensation usually is negligible. These reagents, however, fail to differentiate between many types of compounds producing hydrogen ions. Two acids with widely different ionization constants, such as a strong inorganic acid and a normal carboxylic acid, usually can be preferentially determined, although a potentiometric titration may be required. These differential titrations, however, tend to lose their precision and accuracy when the acid strengths become more nearly equal or when the concentration of one acid is relatively low with respect to the other.

In a limited number of cases oxidation methods have been found useful, particularly for the lower fatty acids. Formic acid, for example, has been determined by oxidation with potassium permanganate,[1] mercuric oxide,[2] mecuric acetate,[3,4] and bromine:[5]

$$HCOOH + Br_2 \longrightarrow 2\,HBr + CO_2 \qquad (1)$$

Some use has been made of Kolthoff's iodometric technique:[6]

$$KIO_3 + 5\,KI + 6\,RCOOH \longrightarrow 3\,I_2 + 3\,H_2O + 6\,RCOOK \qquad (2)$$

in the determination of glycolic, some dibasic,[7] and amino acids.[8]

[1] Hanak, A., and Kürschner, K., *Z. Untersuch. Lebensm.*, **60**, 278–90 (1930).

[2] Osburn, O. L., Wood, H. G., and Werkman, C. H., *Ind. Eng. Chem., Anal. Ed.*, **5**, 247–50 (1933).

[3] Wiehe, H. D., and Jacobs, P. B., *Ibid.*, **8**, 44–7 (1936).

[4] Reid, J. D., and Wiehe, H. D., *Ibid.*, **10**, 271–2 (1938).

[5] Frehden, O., and Fürst, K., *Mikrochemie*, **25**, 256–7 (1938).

[6] Kolthoff, I. M., *J. Am. Chem. Soc.*, **48**, 1447–54 (1926).

[7] Singh, B., and Singh, S., *J. Indian Chem. Soc.*, **16**, 343–5 (1939).

[8] Ruziczka, W., *Z. anal. Chem.*, **126**, 94–7 (1943).

Usually excess sodium thiosulfate was added to the iodate–iodide mixture and, after reaction, the excess was determined by titration with standard iodine solution.

The preferential oxidation of propionic acid to oxalic acid with permanganate has been made the basis of a specific determination for this acid in the presence of acetic and formic acids.[9] In some cases the sulfite addition reaction was used for the estimation of unsaturated acids:[10]

$$RCH{=}CHCOONa + Na_2SO_3 + H_2O \longrightarrow RCH_2CH(SO_3Na)COONa + NaOH \quad (3)$$

A carboxyl procedure, independent of acid-base titrimetry, was reported by Mitchell, Smith, and Bryant,[11] based on the equation:

$$RCOOH + CH_3OH \xrightarrow{BF_3} RCOOCH_3 + H_2O \quad (4)$$

The reaction was identical to that for the determination of alcoholic hydroxyl (Chapter IX). Essentially stoichiometric conversion of a large number of organic acids to the corresponding esters and water was accomplished by the use of a large excess of methanol with boron trifluoride as esterification catalyst. (Nieuwland and his coworkers found that boron trifluoride was an effective esterification catalyst; see Chapter IX.) Since 1 mole of water was formed for each equivalent of carboxyl esterified, the water found on titration with Karl Fischer reagent, less that originally present, was a measure of organic acid. The procedure was unaffected by the presence of easily hydrolyzed esters or inorganic acids (with the exception of sulfuric acid) and was remarkably precise in the presence of large quantities of water. The apparatus requirements were very simple, the entire analysis, including the esterification and titration steps, being carried out in glass-stoppered volumetric flasks.

This esterification procedure was applicable to aliphatic acids generally, including branched chain and hydroxyl substituted types, and also to those aromatic acids having the carboxyl attached to an aliphatic side chain. Aromatic acids having the carboxyl group attached to the ring did not react quantitatively. Amino acids, the heterocyclic furoic acid, and the sterically hindered alicylic camphoric acid also failed to react completely.

---

[9] McNair, J. B., *J. Am. Chem. Soc.*, **54**, 3249–50 (1932).

[10] Rosenthaler, L., *Pharm. Acta Helv.*, **17**, 196–7 (1942).

[11] Mitchell, J., Jr., Smith, D. M., and Bryant. W. M. D., *J. Am. Chem. Soc.*, **62**, 4–6 (1940).

B. Development of the Method

Preliminary experiments, analogous to those involving methanol esterification, were made on the following synthetic mixture:

Substance	Weight per cent	Volume, ml.
Acetic acid	17.7	1.0
Methyl acetate	15.7	1.0
Methanol	13.3	1.0
Water	21.3	2.5 ($BF_3 \cdot 2.5H_2O$)
Boron trifluoride	32.0	
Total	100.0	

When diluted to 250 ml. with methanol and allowed to stand at 25 ± 3°C., this composition formed additional methyl acetate at the expense of acetic acid and methanol, based on titration for water with Karl Fischer reagent.[12] Five milliliter portions were titrated at various time intervals, as shown in Table CVI.

TABLE CVI

Rate of Esterification of Acetic Acid Solution[12]

Time after mixing	Water found, mg.		Acetic acid found,[a] wt. %
	Total	Net (corr. for calculated free water)	
5 minutes	156.6	16.8	9.5
	162.9	23.1	13.1
	164.7	24.9	14.1
1 hour	171.1	31.3	17.7
	170.8	31.0	17.5
2 hours	171.9	32.1	18.2
18 hours	170.2	30.4	17.2
	170.9	31.1	17.6

[a] Calculated 17.7 per cent acetic acid.

Based on these data, the reaction was complete in less than 1 hour at room temperature. For convenience, a catalyst was prepared containing 100 grams of boron trifluoride per liter of methanol solution. Rate studies were made on about 10 milliequivalent portions of lower fatty acids with 10 or 20 ml. of catalyst solution under the conditions shown in Table CVII.

---

[12] Unpublished results from the authors' laboratory.

TABLE CVII

Rates of Reaction of Fatty Acids[12]

Acid	Weight per cent of theoretical carboxyl					
	10 ml. catalyst solution			20 ml. catalyst solution		
	1 hr., R.T.[a]	0.5 hr., 60°C.	1 hr., 60°C.	1 hr., R.T.[a]	2 hrs., R.T.[a]	1 hr., 60°C.
Formic				98.5	99.5	99.5
Acetic	100	100	100	100	100	100
Propionic	100	99	100	100	100	100
n-Butyric		95.5		98.5	99.5	100
Isobutyric		96.5		99	100	100
n-Caproic		94		99	100	100

[a] R.T. = room temperature (27 ± 2°C.).

C. Reagents

The general carboxyl reagent containing about 100 grams of boron trifluoride per liter is prepared by diluting 195 grams (150 ml.) of the $BF_3 \cdot 2CH_3OH$ to 1 liter with methanol.

An esterification reagent also may be prepared from boron trifluoride etherate. Results about 99 per cent quantitative were obtained on acetic and trimethyl acetic acids, using a catalyst containing 37.5 grams of boron trifluoride per 250 ml. (150 grams per liter). The reagent was prepared by diluting about 70 ml. (75.8 grams) of commercial $BF_3 \cdot C_2H_5OC_2H_5$ to 250 ml. with dry methanol. This reagent tended to darken on standing; it was best prepared and used as needed (see page 274, Chapter IX).

Other reagents are technical dioxane and C.P. pyridine (each containing <0.1 per cent water).

The stock catalyst solution, $BF_3 \cdot 2CH_3OH$, is prepared in the same apparatus as the acetic acid reagent (see Chapter IX). About 770 grams (975 ml.) of C.P. methanol are weighed to the nearest gram into a 2 liter three-necked flask. The equipment is assembled as shown in Figure 42, except that in this case the ice bath surrounding the flask is filled immediately. With the stirrer running, the BF_3 cylinder valves are opened carefully and the flow is adjusted so that no vapors escape from the top of the condenser. The absorption of boron trifluoride in alcohol is more rapid than in acetic acid, permitting faster addition. After the contents of a 2 lb. cylinder or its equivalent have been added, the flask is removed and reweighed, the net increase representing the quantity of boron trifluoride absorbed.

Ordinarily about 86 grams (125 ml.) of methanol are required to adjust the concentration to the desired $BF_3 \cdot 2CH_3OH$. The solution is stored in a narrow-mouth, rubber-stoppered bottle. This reagent is not as stable as the acetic acid complex. The methanol is dehydrated slowly to dimethyl ether:

$$2\ CH_3OH \xrightarrow{BF_3} CH_3OCH_3 + H_2O \tag{5}$$

but the reagent can be stored for several months before the water concentration becomes unduly large.

D. General Analytical Procedure

The sample containing up to 160 milliequivalents of acid (total acid plus water should be no more than 300 milliequivalents; larger quantities of water result in excessive titrations) is weighed into a 100 ml. glass-stoppered volumetric flask about one third filled with technical dioxane. The mixture is made up to volume with more dioxane, thermostating (near room temperature) if all aliquots are not to be removed at the same time. Exactly 5 ml. of the dioxane solution is transferred to a 250 ml. glass-stoppered volumetric flask.

If more convenient, the sample containing up to 8 milliequivalents of acid, but no more than 15 milliequivalents of acid plus water, may be weighed directly into the 250 ml. volumetric flask, avoiding the use of dioxane in sample or blank. Solid acids ordinarily are not aliquoted since they usually are not very soluble in dioxane. Where the water content of the sample is known or is determined separately, methanol may be used as a diluent.

After the addition of 20 ml. of catalyst solution, the loosely stoppered flask is placed in a water bath at 60 ± 1°C. The stopper is raised momentarily to allow for expansion of enclosed air, then is firmly stoppered, and the solution is heated for 1 hour. At the end of this period the flask is removed from the bath and allowed to cool spontaneously to room temperature. Then 5 ml. of C.P. dry pyridine are added carefully and the mixture is titrated with Karl Fischer reagent. At least one blank is run containing 20 ml. of catalyst solution, 5 ml. of pyridine, and, if used with the sample, 5 ml. of dioxane.

When pyridine is not used, a false endpoint is obtained after the addition of a few milliliters of Fischer reagent. Unlike the acetic acid reagent for alcoholic hydroxyl, no interfering reaction occurs at this point and the titration can be continued without error. Unless about 50 milligrams of water are present, however, the false endpoint may carry through the true endpoint. The addition of pyridine before titration entirely eliminates this false endpoint; otherwise additional water should be added to the catalyst solution to bring the total quantity in the blanks to about 50 milligrams.

After correction for water in the dioxane, catalyst, and pyridine, the millimoles of water present after treatment, less the water originally present, are equal to the milliequivalents of free carboxyl in the sample.

The water originally present in the sample may be obtained by titrating 5 ml. of the original dioxane solution with Fischer reagent. A correction must be made for the water content of the dioxane itself as this quantity is usually appreciable. The correction is made on the basis of the volume of dioxane in the aliquot, figured with sufficient precision as the difference between the total volume of solution and the volume of concentrated sample employed, neglecting volume changes on mixing.

A typical analysis indicates the data which must be taken: 8.6354 grams of trimethylacetic acid (M.W. = 102.1) were diluted to 100 ml. in dioxane. The 5 ml. aliquots used for analysis contained approximately 0.4 ml. (0.4318 g.) of sample and 4.6 ml. of dioxane:

Solution	Fischer reagent, ml. (0.1566 millimole H_2O/ml.)	Water found, millimoles	
		Gross	Net
5 ml. dioxane	3.2	(*a*) 0.50	(*a*′) 0.46/4.6 ml. dioxane
5 ml. sample aliquot	3.5	(*b*) 0.55	(*b*′) $0.09 = (b - a') \approx 0.4\%$ H_2O
5 ml. dioxane + 20 ml. catalyst[a] (control)	16.3	(*c*) 2.55	(*c*′) $2.5 = (c - a + a')$
5 ml. sample + 20 ml. catalyst[a]	43.5	(*d*) 6.81	(*d*′) $4.21 = [d - (c' + b')]$

$$\frac{4.21 \times 102.1}{1000 \times 0.4318} \times 100 = 99.6\% \text{ trimethylacetic acid}$$

$$99.6\% + 0.4\% = 100.0\% \text{ total}$$

[a] Plus 5 ml. of pyridine.

E. Analytical Results

The experimental results obtained on several C.P. organic acids are given in Table CVIII. The average precision and accuracy based on comparisons with alkali titrations are about ±0.3 per cent.

TABLE CVIII

Analytical Data for Organic Acids[11,12]

Acid		Complete analysis using Karl Fischer reagent, wt. %		
Name	Alkali titration, wt. %	Acid[a]	Water	Total
Formic		(6) 97.2 ± 0.2	2.2	99.4
Acetic		(2) 99.6 ± 0.2	0.3	99.9
Propionic		(2) 99.8 ± 0.1	0.2	100.0
Malonic	97.6	(2) 97.2 ± 0.3	2.1	99.3
n-Butyric	99.5	(8) 99.5 ± 0.4	0.5	100.0
Isobutyric	99.5	(6) 99.7 ± 0.2	0.5	100.2
Succinic		(2) 99.1 ± 0.5	0.7	99.8
dl-Methylethylacetic	95.2	(2) 95.1 ± 0.0	0.7	95.8
Trimethylacetic		(2) 99.6 ± 0.0	0.4	100.0
Glutaric	97.3	(3) 97.7 ± 0.2	0.9	98.6
n-Caproic	99.1	(6) 99.3 ± 0.3	0.4	99.7
Adipic	99.2	(2) 99.0 ± 0.1	0.1	99.1
Lauric	98.6	(2) 98.9 ± 0.1	0.0	98.9
Stearic		(4) 100.3 ± 0.1	0.0	100.3
Oleic	99.5	(2) 99.2 ± 0.0	0.5	99.7
Linoleic	98.2	(2) 96.5 ± 0.5	1.2	97.7
Cyclohexanecarboxylic	98.4	(2) 98.3 ± 0.1	0.1	98.4
Cyclohexylacetic	99.4	(3) 99.3 ± 0.2	0.0	99.3
Phenylacetic		(2) 100.0 ± 0.2	0.0	100.0
Cinnamic		(2) 99.7 ± 0.3	0.0	99.7

[a] Figures in parentheses represent number of individual determinations.

This procedure was applied successfully to a number of complex mixtures. Examples of typical analyses of solutions containing boron trifluoride and hydrogen fluoride are shown in the table below:[12]

Component	Found, wt. %			
	No. 1	No. 2	No. 3	No. 4
Hydrogen fluoride	0.0	0.6	1.4	0.0
Boron trifluoride	57.5	33.1	65.0	59.0
Water	33.0 ± 0.2	20.0 ± 0.1	9.8 ± 0.0	9.6 ± 0.1
Acid[a]	9.0 ± 0.1	27.5 ± 0.2	1.6 ± 0.2	28.2 ± 0.3
Ester[b]	0.0	18.3	6.1	2.8
Alcohol[c]	0.0 ± 0.0	0.2 ± 0.1	16.1 ± 0.1	1.1 ± 0.2
Total	99.5	99.7	100.0	100.7

[a] Acetic or propionic acid.

[b] Methyl acetate or ethyl propionate.

[c] Methanol or ethanol, analysis by hydroxyl procedure of Chapter IX.

The effect of catalyst concentration on camphoric, fencholic, furoic, and some aromatic acids on heating for 1 hour at 60°C. is shown in Table CIX. On substituting glycol in the one case and diisopropylcarbinol in the other for methanol in the catalyst solution and following the other conditions of the general procedure (including boron trifluoride concentration) the extent of reaction with benzoic acid was increased to 75 per cent, with the glycol solution, and decreased to 16 per cent, with the diisopropylcarbinol solution.[11]

TABLE CIX

Esterification Using Higher Concentrations of Catalyst[11,12]

Substance	Per cent acid esterified using methanol containing		
	100 g. BF_3/l.[a]	200 g. BF_3/l.	300 g. BF_3/l.
Benzoic	58	93	98.5 ± 0.3
Salicylic	18	46	67
Anisic			88.5
Orthophthalic	44		88
Terephthalic	10[b]		25[b]
Camphoric	40		46
Furoic	34	52	83
Fencholic	81.9 ± 0.1		

[a] General catalyst.
[b] Sample insoluble in esterification reagent.

A decrease in the catalyst concentration, while having little effect on the esterification of some aliphatic acids, reduced considerably the reaction of aromatic and heterocyclic acids and camphoric acid. Experimental results are noted in Table CX. The ease with which stearic acid esterified appeared to be outstanding. Lauric acid was only 79 per cent esterified after 1 hour at 26°C., using the 25 grams of boron trifluoride per liter catalyst. This C_{12} acid reacted to some extent with less concentrated catalyst solutions, giving values of 70 and 40 per cent with 15 grams of boron trifluoride per liter and 5 grams of boron trifluoride per liter catalysts, respectively. On the assumption that mixtures of aliphatic and aromatic acids would behave in the same manner as the pure substances (see Table CX), known mixtures of acetic–benzoic and acetic–salicylic acids were analyzed in duplicate using the 25 grams of boron trifluoride per liter catalyst and allowing the mixtures to stand for 1 hour at

TABLE CX

Esterification Using Lower Concentrations of Catalyst[11]

Substance	Per cent acid esterified			
	Catalyst, 25 g. BF_3/l.		Catalyst, 50 g. BF_3/l.	
	1 hr., R.T.[a]	1 hr., 60°C.	1 hr., R.T.[a]	1 hr., 60°C.
Formic	96.9 ± 0.1	97.7 ± 0.0	97.7 ± 0.0	
Acetic	98.8 ± 0.0	98.8 ± 0.0	99.1 ± 0.2	99.8 ± 0.1
Isobutyric	96.2 ± 0.0	99.0 ± 0.2	98.9 ± 0.2	
Trimethylacetic	31.7 ± 0.0	95.8 ± 0.3	52.9 ± 0.0	96.5 ± 0.3
n-Caproic	98.6 ± 0.0			
Oleic	95.6 ± 0.0			
Stearic	100.3 ± 0.0			
Succinic	87.6 ± 0.6	99.1 ± 0.3		
Camphoric	1.3 ± 0.5			
Benzoic	1.4 ± 0.8	34.9 ± 0.3	6.2 ± 0.0	52.5 ± 0.3
Salicylic	0.0 ± 0.6	8.4 ± 0.0	1.1 ± 0.5	18.4
Furoic	0.9 ± 0.3			

[a] R.T. = room temperature (26 ± 1°C.).

TABLE CXI

Analyses for Acetic Acid in Presence of Benzoic and Salicylic Acids[11]

Nature of mixture	Aromatic acid added, wt. %	Acetic acid added, wt. %	Acetic acid[a] found, wt. %
Acetic and benzoic acids	89.6	10.4	12.6 ± 0.0
	50.8	49.2	50.1 ± 0.1
	11.8	88.2	88.1 ± 0.1
Acetic and salicylic acids	91.3	8.7	9.0 ± 0.0
	47.1	52.9	52.9 ± 0.0
	11.7	88.3	87.7 ± 0.1

[a] No corrections applied for reactivities according to Table CX.

room temperature. These results are given in Table CXI. These data should be representative of the behavior to be expected with many aliphatic–aromatic acid mixtures. The procedure with dilute catalyst might be used for the analysis of other mixtures, taking into account the behavior of the pure acids on the basis of Table CX.

F. Interfering Substances

In the study of possible interfering classes of compounds it was found that esters, acetals, amides, nitriles, unsaturated alcohols, and unsaturated hydrocarbons did not react.[11] Under the conditions of the general procedure (100 grams of boron trifluoride per liter catalyst, 1 hour at 60°C.) results of 0.0 per cent were obtained on methyl formate, acetal, di-*n*-butyl formal, methylal, formamide, acetonitrile, adiponitrile, allyl alcohol, and amylene. This lack of interference was further demonstrated in the analysis of carboxylic acids containing other functional groups, examples of which are given in Table CXII.

TABLE CXII

Analytical Data for Carboxylic Acids Containing Other Functional Groups[11,12]

Acid		Complete analysis using Karl Fischer reagent, wt. %		
Name	Alkali titration, wt. %	Acid[a]	Water	Total
Glycolic	99.9	(4) 99.6 ± 0.2	0.0	99.6
α-Hydroxyisobutyric	97.6	(4) 97.9 ± 0.5	0.1	98.0
Tartaric	94.5	(4) 94.3 ± 0.5	0.1	94.4
Ricinoleic[b]	58.3	(4) 58.4 ± 0.5	1.8	60.2
Monochloroacetic	98.8	(3) 99.0 ± 0.2	0.2	99.2
Dichloroacetic	99.0	(2) 98.8 ± 0.2	0.2	99.0
Trichloroacetic	93.6	(4) 91.5 ± 0.2	0.6	92.1
Diglycolic		(4) 93.2 ± 0.3	6.4	99.6
α,α′-Thiodiglycolic	96.9	(2) 96.9 ± 0.1	1.3	98.1
β,β′-Thiodipropionic	99.0	(2) 100.6 ± 0.2	0.2	100.8
2,4-Dichlorophenoxyacetic	99.0	(2) 99.0 ± 0.0	0.5	99.5

[a] Figures in parentheses represent number of individual determinations.
[b] Technical grade, all others C.P. quality.

Some classes of organic acids reacted incompletely by this procedure. Benzilic acid, $(C_6H_5)_2C(OH)COOH$, which should esterify as an aliphatic acid, reacted only 45 per cent, while citric acid, $HOOCCH_2C(OH)(COOH)CH_2COOH$, was about 92 per cent esterified.

Amino acids did not react completely under the conditions of the general method, possibly due to the inhibiting action of the amine group on the catalyst. In some cases an increase in catalyst concentration to 300 grams of boron trifluoride per liter of methanol solution was sufficient to effect nearly quantitative esterification after 1 hour at 70°C.[12] Results obtained on a few amino acids, using 20 ml. of reagent, are given in Table CXIII.

TABLE CXIII

Esterification of Amino Acids[12]

Substance	Amount, mM[a]	Solvent	Catalyst, g.BF_3/l.	Conditions	Per cent reaction
Glycine	10	Dioxane	100	1 hr., 60°C.	30
	5	Dioxane	200	1 hr., 65°C.	87
	10	Dioxane	200	1 hr., 65°C.	80
	5	Dioxane	300	1 hr., 65°C.	97.2 ± 0.1
	10	Dioxane	300	1 hr., 65°C.	95.3
	10	Dioxane	300	2 hr., 65°C.	100.0 ± 0.3
	10	Dioxane	300	1 hr., 70° C.	99.8 ± 0.2
	10	Dioxane	300	2 hr., 70°C.	98
	10	None	300	1 hr., 70°C.	100.0 ± 0.5
Glutamic acid	10	None	300	1 hr., 70°C.	99.0 ± 0.5
Leucine	10	None	300	1 hr., 70°C.	92.7
Iminodiacetic acid	10	None	100	1 hr., 60°C.	44
	10	None	300	1 hr., 70°C.	92
Betaine hydrochloride	10	None	300	1 hr., 70°C.	82
Alanine	10	None	300	1 hr., 70°C.	96.7 ± 0.2
Nitrilotriacetic acid	10	None	100	1 hr., 60°C.	58.5

[a] Millimoles.

No interference was encountered with benzene sulfonic, phosphoric, and hydrochloric acids. Sulfuric acid, however, was esterified by the catalyst solution:[11]

$$H_2SO_4 + CH_3OH \xrightarrow{BF_3} CH_3HSO_4 + H_2O \qquad (6)$$

With the 100 grams of boron trifluoride per liter catalyst, this reaction was about 25 per cent complete after 1 or 2 hours at room temperature, but quantitative after 1 or 2 hours at 60°C. Boric acid reacted stoichiometrically with the methanol in Karl Fischer reagent, even in the absence of a solvent, forming 3 moles of water per mole of acid[11] (Chapter VIII).

Aldehydes and ketones reacted with the methanol of the catalyst solution presumably to form acetals and ketals:[11]

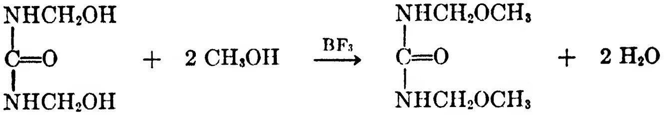
$$RCHO + 2\,CH_3OH \longrightarrow RCH(OCH_3)_2 + H_2O \qquad (7)$$

$$RCOR' + 2\,CH_3OH \longrightarrow RR'C(OCH_3)_2 + H_2O \qquad (8)$$

The reaction with all aldehydes tried (formaldehyde, 2-ethyl-1-hexanal, butyraldehyde) was stoichiometric, so that suitable corrections could be made. Using the general procedure, the reaction of cyclohexanone was essentially complete (95 per cent); other ketones, however, reacted only partially. Acetone and ethyl isopropyl ketone gave values of 48 and 13 per cent, respectively.

Attempts to combine carbonyl compounds as the cyanohydrins by reaction with hydrogen cyanide in dioxane (see Chapter V) prior to the addition of the boron trifluoride–methanol reagent were unsuccessful. Although the carbonyl group was stabilized effectively, subsequent esterification of the organic acid was incomplete. A 75 per cent butyric acid–25 per cent butyraldehyde solution analyzed 65 per cent acid after this treatment.[12] Attempts to remove the carbonyl compound as oxime also were unsuccessful. A 50–50 acetone–acetic acid solution first was treated with hydroxylamine hydrochloride, according to the general carbonyl procedure (see Chapter XII), then the 100 grams of boron trifluoride per liter methanol reagent was added, and the mixture was heated for 1 hour at 60°C. Correcting for the quantitative carbonyl reaction, in which 1 mole of water was formed per mole of acetone, the net water found was equivalent to 40 per cent acetic acid based on the original sample.[12]

Dimethylolurea reacted, apparently with the methanol of the carboxyl catalyst, possibly by way of further condensation:



$$\begin{array}{c}NHCH_2OH\\ |\\ C{=}O\\ |\\ NHCH_2OH\end{array} + 2\,CH_3OH \xrightarrow{BF_3} \begin{array}{c}NHCH_2OCH_3\\ |\\ C{=}O\\ |\\ NHCH_2OCH_3\end{array} + 2\,H_2O \qquad (9)$$

Under the conditions of the general analysis, the net millimoles of water found after reaction were nearly equivalent to the millimoles of formaldehyde present in the sample, as shown in Table CXIV.

TABLE CXIV

Reaction of Dimethylolurea in Carboxyl Reagent[12]

Sample No.	Urea, %	Formaldehyde, %	Formaldehyde taken, mM[a]	Water found, mM[a]	Ratio, water: formaldehyde
1	57.96	41.44	13.81	13.59	0.98
2	50.14	49.11	16.37	16.16	0.99
3	49.16	50.38	16.38	15.44	0.94

[a] Millimoles.

Anhydrides also interfered with the procedure, probably as follows:[11]

$$(RCO)_2O + CH_3OH \longrightarrow RCOOCH_3 + RCOOH \qquad (10)$$

$$RCOOH + CH_3OH \longrightarrow RCOOCH_3 + H_2O \qquad (11)$$

On this basis, using the general procedure, the extent of reaction with aliquots of acetic anhydride dissolved in dioxane was about 61 per cent.[11] In methanol soultion, however, the reaction with aliphatic anhydrides in general was essentially quantitative, as indicated in Table CXV. These data are compared with corresponding results by the sodium hydroxide–sodium methylate procedure of Smith and Bryant.[13] The correction for free acid to be applied to the Fischer reagent procedure was calculated from the acidimetric titration data. As expected, the aromatic and heterocyclic anhydrides failed to react completely.

TABLE CXV

Reaction of Anhydrides with Carboxyl Catalyst[12]

Substance	Anhydride found, wt. %	
	BF_3-CH_3COOH procedure	NaOH-$NaOCH_3$ procedure
Acetic	100.3 ± 0.3	99.9
Propionic	97.1 ± 0.3	97.2
Succinic	97.4 ± 0.2	97.5
Maleic	89.4 ± 0.2	90.0
Benzoic	78.0 ± 0.4	99.2
Phthalic	51.2 ± 0.2	99.0
Furoic	72.2 ± 0.5	94.8

---

[13] Smith, D. M., and Bryant, W. M. D., *J. Am. Chem. Soc.*, **58**, 2452–4 (1936).

CHAPTER XI

Determination of Acid Anhydrides

A. Introduction

Many of the older methods of determining anhydrides were based on hydrolysis to the free acid followed by titration with caustic. These techniques failed to distinguish sharply between anhydride and free acidity. The same was true of procedures employing esterification or amination.[1] Lukashevich[2] suggested a combination hydrolysis–acylation technique:

$$(RCO)_2O + H_2O \longrightarrow 2\,RCOOH \qquad (1)$$

$$(RCO)_2O + CH_3OH \longrightarrow RCOOCH_3 + RCOOH \qquad (2)$$

One portion of the sample was boiled with excess water and a second portion with methanol. The difference between the titrations for free acidity was equivalent to anhydride. In the absence of an esterification catalyst, free acids did not interfere since they titrated mole for mole with the caustic.

Smith and Bryant[3] suggested an improved two-step technique in which the reactions were completed rapidly at room temperature. A weighed quantity of the sample was hydrolyzed immediately at room temperature, using excess water with pyridine as a catalyst, followed by titration with standard aqueous sodium hydroxide. A second sample was titrated directly with alcoholic sodium methylate:

$$(RCO)_2O + CH_3ONa \longrightarrow RCOOCH_3 + RCOONa \qquad (3)$$

The mild conditions required for this analysis together with the pyridine tended to discourage any esterification of free acids with the methanol of the reagent and consequently the procedure was generally applicable to the determination of anhydrides in the presence of free acids. The method, however, became less accurate for

---

[1] Malm, C. J., and Nadeau, V., United States Patent 2,063,324 (1936).

[2] Lukashevich, V. D., *J. Chem. Ind. USSR*, **8**, 1086–7 (1931).

[3] Smith, D. M., and Bryant, W. M. D., *J. Am. Chem. Soc.*, **58**, 2452–4 (1936).

samples containing only small quantities of anhydride in acid solutions and was subject to interference by easily hydrolyzed esters and by certain acidic inorganic compounds, particularly those too weak to titrate completely.

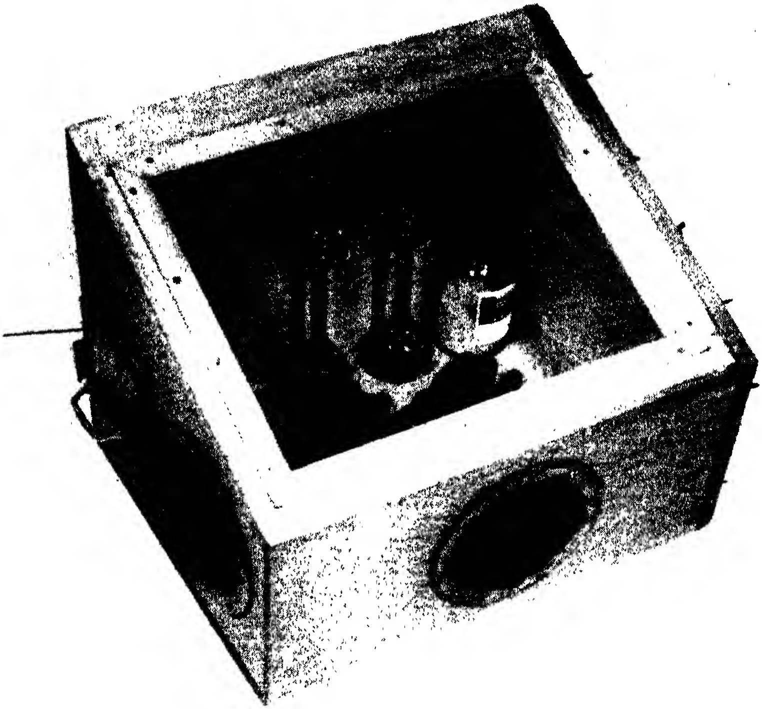


Fig. 44. "Dry manipulator" for transferring hygroscopic materials.[6]

Two hydrolysis techniques, independent of acidimetry, were reported by Smith, Bryant, and Mitchell.[4,5] The one depended on hydrolysis of the anhydride in acetic acid solution under the influence of boron trifluoride[4] and the other, a more widely applicable technique, was based on hydrolysis in pyridine solution using so-

---

[4] Smith, D. M., Bryant, W. M. D., and Mitchell, J., Jr., *J. Am. Chem. Soc.*, 62, 608–9 (1940).

[5] Smith, D. M., Bryant, W. M. D., and Mitchell, J., Jr., *Ibid.*, 63, 1700–1 (1941).

[6] Unpublished results from the authors' laboratory.

dium iodide as catalyst.[5] In both cases, the water used up was determined by titration with Fischer reagent of the solutions after hydrolysis. Unlike their behavior in acidimetric analyses, free acids did not interfere and easily hydrolyzed esters remained unchanged due to the relatively low water concentrations of the reagents in these procedures.

Solid anhydrides tended to adsorb moisture on exposure to the atmosphere. In order to prevent this, a "dry manipulator" was used to dispense these materials. With this unit, shown in Figure 44, an anhydrous atmosphere was maintained by forced circulation of dry air. The large rubber gloves permitted manipulations from the outside which were observed through the plate glass window. A small spring-clip door on one side allowed the rapid transfer of small equipment, flasks, etc. The opposite side could be removed completely for larger apparatus; for example, the box accommodated a torsion balance of 120 grams capacity in each pan.[6]

B. Development of Acid Hydrolysis Procedure

In the initial experiments, 5 ml. samples of propionic anhydride in dioxane solution (2.1 millimoles per 5 ml.) were treated with 20 ml. of a boron trifluoride–acetic acid catalyst (100 grams of boron trifluoride per liter) containing about 7.5 millimoles of water; the anhydride was hydrolyzed to the extent of 93.5, 98.5, and 99.8 per cent after 1 hour at room temperature and 1 and 2 hours at 60°C., respectively. Several other anhydrides were studied, using 5 ml. aliquots from dioxane solutions and heating for 2 hours at 60°C. Results are noted in Table CXVI.

TABLE CXVI

Acid Hydrolysis of Anhydrides in Dioxane Solution[6]

Anhydride	Millimoles		Hydrolysis, %
	Sample	Water added	
Acetic	2.62	7.63	100
Propionic	2.10	7.50	99.8
n-Butyric	1.73	7.63	99
Crotonic	2.01	7.63	100
Caproic	1.92	7.63	95
Heptylic	1.89	7.61	94
Succinic	2.05	7.61	90

The tendency toward low results with the 100 grams of boron trifluoride per liter reagent was reduced when these anhydrides were either aliquoted from acetic acid (rather than dioxane) solution or weighed directly. Changes in catalyst concentration had marked effects on the rates of hydrolysis of anhydrides. About 5 millimole quantities of a few anhydrides in acetic acid solution were treated with 20 ml. portions of catalyst solutions containing various concentrations of boron trifluoride and heated for 2 hours at 60°. The results are given in Figure 45. The activity of the anhydrides derived from dibasic acids was interesting. With the exception of glutaric, those anhydrides studied showed maximum reactions when relatively small quantities of boron trifluoride were present.[3]

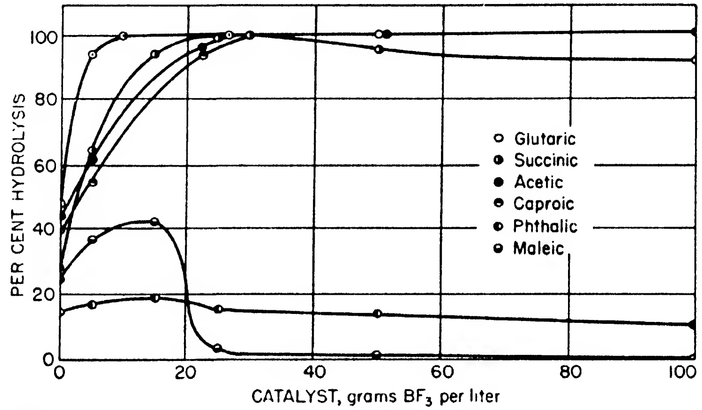


Fig. 45. Acid hydrolysis of anhydrides using catalyst solutions of various concentrations.[4]

From an examination of Figure 45, it is evident that an approximate procedure could be devised for distinguishing between certain anhydrides. For such analyses, control experiments should be run as a means of estimating the probable errors.

It was shown in Chapter V that the rate of hydrolysis of anhydrides was relatively slow in the absence of a catalyst or acid acceptor. Therefore, unless the sample to be analyzed was known to be anhydrous, a titration for free water was made at the time of analysis. The quantity found was added to the blank titer, since this represented additional water not present in the blank.

C. Reagents

The catalyst solution is prepared by diluting 210 ml. (278 grams) of $BF_3 \cdot 2CH_3COOH$ plus about 6.5 ml. of water to 1 liter with C.P. glacial acetic acid; C.P. glacial acetic acid and C.P. pyridine (each <0.1 per cent water) also are used. (See page 275, Chapter IX, for preparation of $BF_3 \cdot 2CH_3COOH$.)

The catalyst solution has the same concentration of boron trifluoride as that used for the determination of alcoholic hydroxyl, namely, 100 grams per liter. In this procedure, however, the reagent also contains sufficient water to insure about a 100 per cent excess over that required for complete hydrolysis of the anhydride.

D. Acid Hydrolysis Procedure

The sample containing up to 4 millimoles of anhydride is weighed or transferred into a 250 ml. glass-stoppered volumetric flask. (The sample may either be weighed directly or a 5 ml. portion aliquoted from acetic acid solution.) Twenty milliliters of the catalyst solution are added. This flask and a control containing 20 ml. of catalyst solution (and 5 ml. of acetic acid if an acetic acid solution of the sample was used) are placed in a water bath at 60 ± 1°C. The stoppers are loosened momentarily to allow for expansion of included air and are then tightened; the flasks are heated for 2 hours. After the vessels have cooled spontaneously to room temperature, 5 ml. of C.P. pyridine are added carefully to each and the excess water is titrated with Karl Fischer reagent. The total anhydride is measured by the difference between water added, i.e., the adjusted water content of the blank, and the water found by titration after reaction.

Free water in the sample is determined by direct titration of a suitable aliquot of the acetic acid solution immediately after dilution, otherwise, on standing, measurable quantities of water may be absorbed by the anhydride. If desired, however, the aliquot may be kept in a chilled glass-stoppered flask until the other titrations are made, since at low temperatures (0°C. or below) in the absence of a catalyst the reaction of anhydrides with water generally is negligible.[5] Free water in the sample is added to the blank titer.

A typical analysis was calculated as follows: 5.3484 grams of acetic anhydride (M.W. = 102.1) were diluted to exactly 100 ml. with glacial acetic acid. The 5 ml. portion used for analysis contained 0.2674 gram (2.62 millimoles, 0.3 ml.) of sample and 4.7 ml. of acetic acid.

Solution	Water found, millimoles	
	Gross	Net
5 ml. acetic acid	(*a*) 1.39	(*a*′) 1.31/4.7 ml. CH_3COOH
5 ml. sample aliquot	(*b*) 1.31	(*b*′) $0.0 = (b - a') \approx 0.0\%$ H_2O
5 ml. acetic acid + 20 ml. catalyst	(*c*) 7.65	(*c*′) $7.57 = [c - (a - a')]$
5 ml. aliquot + 20 ml. catalyst	(*d*) 4.97	(*d*′) $2.60 = (c' - d)$

$$\frac{2.60 \times 102.1}{1000 \times 0.2674} \times 100 = 99.4\% \text{ acetic anhydride}$$

E. Experimental Results

A group of anhydrides were analyzed by this procedure and checked by the sodium hydroxide–sodium methylate method.[8] The precision and accuracy usually were within ±0.3 per cent. Experimental results are given in Table CXVII. Although glutaric anhydride hydrolyzed quantitatively, other anhydrides derived from dibasic acids failed to react completely. Results with maleic, phthalic, and succinic anhydrides showed 0, 11, and 91 per cent hydrolysis, respectively (see Fig. 45).

TABLE CXVII

Analytical Data for Anhydrides by Acid Hydrolysis[4]

Anhydride	Anhydride found, wt. %	
	Catalytic hydrolysis[a]	Sodium hydroxide–sodium methylate
Acetic	(6) 100.0 ± 0.2	99.9
Propionic	(4) 97.0 ± 0.3	97.2
n-Butyric	(2) 85.5 ± 0.5	85.4
Crotonic	(2) 89.6 ± 0.0	
n-Valeric[b]	(2) 2.0 ± 0.4	1.9
Caproic	(2) 78.6 ± 0.4	78.4
Heptylic	(2) 92.0 ± 0.3	92.1
Glutaric	(2) 95.3 ± 0.3	95.5
Benzoic	(4) 99.0 ± 0.3	99.2
Furoic	(4) 95.1 ± 0.5	94.8

[a] Figures in parentheses represent number of individual determinations.

[b] Old sample, mainly *n*-valeric acid, used to test method in low anhydride range.

The procedure was adversely affected by those compounds which interfered with the general hydroxyl procedure (Chapter IX) and included aldehydes, cyclohexanone, acetals, and ortho esters.

F. Development of the Alkaline Hydrolysis Procedure

The second and more widely applicable procedure for the determination of anhydrides using Fischer reagent was based on hydrolysis in the presence of pyridine, using sodium iodide as catalyst, according to the equation:

$$(RCO)_2O + H_2O \xrightarrow[C_5H_5N]{NaI} 2\,RCOOH \qquad (4)$$

The excess water after reaction was determined by titration with Karl Fischer reagent.

It seemed possible that hydrolysis of acid anhydrides in the presence of slightly more than the theoretical quantity of water would be complete where large excesses of organic bases were used. Several anhydrides were treated directly with 25 ml. of pyridine of known water content. After standing, as indicated in Table CXVIII,

TABLE CXVIII

Hydrolysis of Anhydrides with Aqueous Pyridine[6]

No.[a]	Anhydride	Millimoles		Conditions	Per cent reaction
		Sample	Total water added		
1	Acetic	96.2	111.2	1 hr., 27°C.	100.0
2		48.0	55.5	1 hr., 27°C.	100.0
3		63.1	74.6	1 hr., 27°C.	98.0
4		38.7	45.3	1 hr., 27°C.	98.5
5		55.2	67.0	1 hr., 60° C.	99.2
6		84.6	96.2	2 hrs., 60°C.	99.6
7		18.2	29.7	2 hrs., 100°C.	100.0
8	Propionic	34.8	55.4	1 hr., 27°C.	86
9		14.3	16.6	2 hrs., 100°C.	97
10	Butyric	25.7	55.4	1 hr., 27°C.	81
11	Crotonic	26.0	55.4	1 hr., 27°C.	73
12		12.8	16.2	2 hrs., 100°C.	86
13	Caproic	17.5	55.4	1 hr., 27°C.	85
14		8.9	15.3	2 hrs., 100°C.	96
15	Heptylic	15.6	55.4	1 hr., 27°C.	89
16		16.1	31.7	1 hr., 60°C.	98
17	Camphoric	13.2	36.1	1 hr., 100°C	5
18	Phthalic	20.2	26.3	1 hr., 60°C.	99

[a] Samples 1–7, 9, 12, 14, 16, and 18 titrated directly; remainder diluted to 100 ml. with pyridine after given time interval and 10 ml. portions titrated for water.

the solutions were titrated for water with Karl Fischer reagent. Water used up was calculated as anhydride and compared with the analyses given by the sodium hydroxide–sodium methylate procedure.[3]

These data indicated that with pyridine alone the hydrolysis of anhydrides in general was incomplete, even though in some cases more than 100 per cent excess of water was present. The ratio of water to anhydride did not appear to be as important a factor as temperature. Since the maximum practical temperature (and time) was included in these experiments, it was concluded that stronger organic amines should be tried as catalysts for the hydrolysis. Results obtained under various conditions are given in Table CXIX. In these experiments, 10 ml. of acetic acid were added to each sample to combine with the excess amine just before titration with Fischer reagent.

TABLE CXIX

Hydrolysis of Anhydrides in Presence of Organic Amines[6]

Anhydride	Millimoles		Amine	Conditions	Per cent reaction
	Sample	Water			
Acetic	18.6	26.8	5 ml. TB^a	1 hr., 60°C.	97
	20.0	28.9	5 ml. TB	3 hrs., 60°C.	97
Crotonic	13.6	22.2	5 ml. TB	3 hrs., 60°C.	98.5
Caproic	8.0	21.5	5 ml. TB	3 hrs., 60°C.	99.5
Lauric	5.2	18.6	5 ml. TB	3 hrs., 60°C.	55
Maleic	12.6	20.3	5 ml. TB	3 hrs., 60°C.	93.5
Succinic	19.6	24.2	5 ml. TB	3 hrs., 60°C.	93.5
	16.8	23.6	10 ml. TB	3 hrs., 60°C.	93
	21.2	30.2	10 ml. TB	2 hrs., 60°C. + 1 hr., 90°C.	93.5
	19.6	28.9	10 ml. TB	3 hrs., 90°C.	95
Camphoric	11.3	22.1	5 ml. TB	3 hrs., 60°C.	15
Benzoic	9.6	20.0	5 ml. TB	3 hrs., 60°C.	92
Phthalic	12.3	23.2	5 ml. TB	3 hrs., 60°C.	101
Furoic	8.1	22.4	5 ml. TB	3 hrs., 60°C.	84
Propionic	16.3	23.0	5 ml. TE^b	1 hr., 27°C.	90
	14.4	20.0	5 ml. TE	1 hr., 60°C.	93
	15.3	23.0	5 ml. TE	2 hrs., 60°C.	97.5
	15.5	24.6	10 ml. TE	1 hr., 27°C.	85.5
	14.3	23.8	10 ml. TE	1 hr., 60°C.	94
	16.5	24.6	10 ml. TE	2 hrs., 60°C.	95
Propionic	15.1	22.5	5 ml. nB^c	1 hr., 60°C.	84
	15.8	22.8	5 ml. nB	2 hrs., 60°C.	99.5
	15.1	23.8	5 ml. nB	3 hrs., 60°C.	100.5
	14.4	20.8	5 ml. nB	4 hrs., 60°C.	101

[a] Tributylamine. [b] Triethylamine. [c] *n*-Butylamine.

A comparison of Tables CXVIII and CXIX indicated that hydrolysis in general was more nearly complete when the stronger bases were used in place of pyridine. The reactions, however, were not generally quantitative and did not offer a basis for a practical technique.

Additional experiments employing both pyridine and tributylamine either together or stepwise (i.e., first treating the sample with pyridine and heating for periods up to 2 hours at 60°C. and then adding tributylamine and heating again) did not effect any improvement. Similar experiments employing these amines with sodium methylate as an acid acceptor tended to show slight improvement.

In simultaneous experiments 2 samples of acetic anhydride in 25 ml. of pyridine and in 25 ml. of pyridine plus 25 ml. of dioxane gave results of 99 and 51 per cent, respectively, after 1 hour at 60°C. The same trend was noticed when tributylamine was substituted for pyridine. Other inert solvents probably would have had effects similar to that of dioxane.

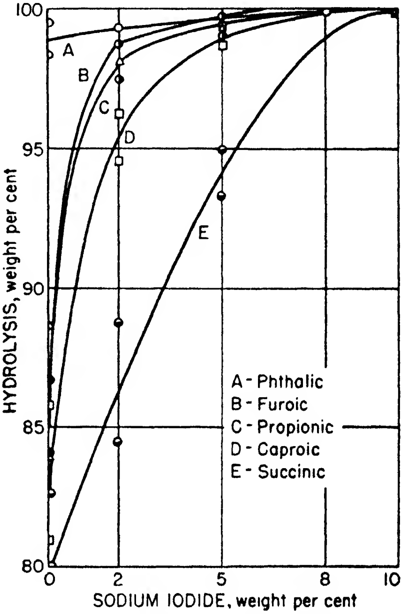


Fig. 46. Effect of concentration of sodium iodide on hydrolysis of anhydrides.[5]

It seemed possible that a basic catalyst effective in the presence of excess amine might be found. Sodium iodide was observed to be quite soluble in pyridine and also inert toward Karl Fischer reagent.[5] Twenty-five milliliter volumes of 1 per cent water-in-pyridine containing various concentrations of sodium iodide, were added to five different types of anhydrides and heated for 1 hour at 60°C. A 40–60 per cent excess of water was employed. The extent of hydrolysis is shown in Figure 46. In all cases, the reaction was quantitative in the presence of 10 per cent sodium iodide in the pyridine. It is interesting to note that hydrolysis of phthalic anhydride was 99 per cent complete even without sodium iodide as was the hydrolysis of acetic anhydride[5] (Table CXVIII). Most other anhydrides, however, required between 45 minutes and 1 hour at 60°C. for complete reaction, using 10 per cent sodium iodide in pyridine reagent.[6]

The type of catalysis involved in the presence of sodium iodide was not known but the high electrical conductivity of sodium iodide in pyridine observed in the authors' laboratory might have some bearing upon its catalytic activity.

G. Reagents for Alkaline Hydrolysis

The alkaline catalyst solution is prepared by dissolving 100 grams of C.P. sodium iodide and 8 ml. of water in C.P. pyridine and diluting to 1 liter with additional pyridine. Normally, C.P. pyridine contains about 0.2 per cent water equivalent to 2 grams per liter. Therefore, only about 8 ml. of additional water is necessary to prepare the required 1 per cent concentration.

H. Alkaline Hydrolysis Procedure

A sample containing up to 10 millimoles of anhydride is weighed into a 250 ml. glass-stoppered volumetric flask. (If more convenient, a 5 ml. aliquot from pyridine solution may be used.) Then 25 ml. of the pyridine reagent are added.

For the analysis, 25 ml. of the reagent, which contains about 14 millimoles of water, is used for a sample containing up to 10 millimoles of anhydride. Thus approximately 40 per cent excess of water is sufficient for complete hydrolysis as compared to the 100 per cent excess recommended for acid hydrolysis.

The flask, together with a blank containing 25 ml. of the reagent (plus 5 ml. of pyridine if a sample aliquot was used), is placed in a water bath at 60 ± 1° C. The stoppers are loosened momentarily (to allow for expansion of included air), then tightened and the flasks are heated for 1 hour. After being allowed to cool spontaneously to room temperature, the excess water is titrated with Karl Fischer reagent. The total anhydride is measured by the difference between water added (adjusted blank titer including free water in the sample) and water found by titration after reaction.

The same precautions for the determination of free water in the sample are observed in this procedure as was indicated for the acid hydrolysis method (see page 315, this chapter).

The procedure for calculation of results is identical to that used for the acid hydrolysis method although different solvents and reagents are used (see page 315, this chapter).

I. Experimental Results

Eleven anhydrides were analyzed by this alkaline hydrolysis method and the results checked by either the sodium hydroxide-sodium methylate[3] or acid hydrolysis procedures.[4] The precision and accuracy usually were within ±0.3 per cent. Experimental results are given in Table CXX.

TABLE CXX

Analytical Data for Anhydrides by Alkaline Hydrolysis[5]

Anhydride	Anhydride found, wt. %			
	Sodium iodide catalyzed hydrolysis[a]		Acid hydrolysis	Sodium hydroxide-sodium methylate
Acetic	(8)	99.5 ± 0.4	97.2	99.5
Propionic	(4)	97.4 ± 0.2	97.2	
n-Butyric	(2)	98.3 ± 0.3		98.3
Crotonic	(2)	87.3 ± 0.1	87.1	
Caproic	(4)	95.0 ± 0.2		94.8
Heptylic	(4)	89.1 ± 0.3	89.2	
Succinic[b]	(4)	91.0 ± 0.5		90.9
Glutaric	(2)	100.1 ± 0.2	100.0	
Benzoic	(5)	89.7 ± 1.0		89.8 ± 0.5
Phthalic[b]	(2)	98.9 ± 0.2		98.4
Furoic	(2)	94.9 ± 0.2	94.9	

[a] Figures in parentheses represent number of individual determinations.
[b] Gave incomplete reaction by the acid hydrolysis method.

Most anhydrides derived from dibasic acids reacted quantitatively, whereas, of the group of four examined, only the glutaric derivative gave stoichiometric results by the acid hydrolysis procedure. Maleic anhydride could not be analyzed by the alkaline hydrolysis method. It reacted violently with the pyridine reagent, probably combining with the pyridine by a Diels-Alder mechanism. Camphoric was the only other anhydride tried which failed to react completely. Probably because of steric hindrance, hydrolysis was only about 1 per cent complete. The crystals of benzoic anhydride used were coated with benzoic acid, as was apparent on optical examination. This almost certainly explains the relatively poor precision obtained on the 5 determinations recorded.[5]

It was evident, from Figure 46 and Table CXX, that the alkaline hydrolysis technique was more widely applicable than the acid hydrolysis method. There was no interference from easily hydrolyzed esters or from low concentrations of most acidic inorganic compounds with either procedure. Zero per cent reactions were obtained on methyl formate, methyl acetate, and methyl propionate. Samples containing high concentrations of inorganic acidic compounds were best analyzed by the boron trifluoride–acetic acid technique since appreciable quantities of such compounds might affect catalyst activity in the sodium iodide–pyridine method.

The alkaline hydrolysis technique was not affected seriously by carbonyl compounds, at least in favorable cases.[6] This effect was demonstrated in the analysis of complex aldehyde–anhydride mixtures. For example, a solution of 25 per cent water, 13 per cent acetaldehyde, 22 per cent acetic anhydride, and 40 per cent acetic acid was prepared and maintained at less than 0°C. in order to minimize hydrolysis prior to analysis. Analytical results were 23.6 ± 0.0 per cent water (as determined by the cyanohydrin method (page 151, Chapter V), 20.0 ± 0.1 per cent acetic anhydride (by the sodium iodide procedure modified in that the sample was allowed to stand for 1 hour at room temperature* followed by the cyanohydrin reaction to combine the aldehyde before titrating with Fischer reagent), and 13.3 per cent acetaldehyde (by the Karl Fischer hydroxylamine procedure (Chapter XII, after first hydrolyzing the anhydride by the sodium iodide–pyridine technique). The lowered results for both water and anhydride suggested partial hydrolysis of the latter prior to analysis.[6]

---

*An anhydride which required heating for complete hydrolysis might have shown more aldehyde interference.

CHAPTER XII

Determination of Carbonyl Compounds

A. Methods of Analysis for Carbonyl Compounds

The most extensive reports on the determination of individual carbonyl compounds have been concerned with methods for estimating formaldehyde. This aldehyde has been determined by a large variety of techniques, exclusive of the many colorimetric methods which were designed primarily for trace analyses. Romijn[1] proposed an iodimetric method:

$$HCHO + NaOI + NaOH \longrightarrow HCOONa + NaI + H_2O \qquad (1)$$

After acidification, unused iodine was determined by titration with thiosulfate. The method has been used widely for specific problems.[2-4] Ripper[5] and others[6-8] studied the bisulfite addition reaction:

$$HCHO + NaHSO_3 \longrightarrow CH_2(OH)SO_3Na \qquad (2)$$

Excess bisulfite was determined by titration with iodine. When sulfite was substituted for bisulfite, a direct acidimetric titration could be used:[9-11]

$$HCHO + Na_2SO_3 + H_2O \longrightarrow CH_2(OH)SO_3Na + NaOH \qquad (3)$$

Other volumetric methods based on specific reactions included ammonia:[12,13]

---

[1] Romijn, G., *Z. anal. Chem.*, **36**, 18–24 (1897).

[2] Hatcher, W. H., and Mueller, W. H., *Trans. Roy. Soc. Canada,* **23**, 35–44 (1929).

[3] Weinberger, W., *Ind. Eng. Chem., Anal. Ed.*, **3**, 357–8 (1931).

[4] Korenman, I. M., *J. Applied Chem. USSR,* **8**, 1476–7 (1935).

[5] Ripper, M., *Monatsh.*, **21**, 1079–85 (1901).

[6] Joslyn, M. A., and Comar, C. L., *Ind. Eng. Chem., Anal. Ed.*, **10**, 364–6 (1938).

[7] Donnally, L. H., *Ibid.*, **5**, 91–2 (1933).

[8] Parkinson, A. E., and Wagner, E. C., *Ibid.*, **6**, 433–6 (1934).

[9] Lumiere, A. L., and Seyewetz, A., *Bull. soc. chim.*, (3), **27**, 1212–4 (1902).

[10] Seyewetz, A., and Gibello, A., *Ibid.*, (3), **31**, 691–4 (1904); *Compt. rend.*, **138**, 1225–7 (1904).

[11] Feinberg, B. G., *Am. Chem. J.*, **49**, 87–116 (1913).

[12] Legler, L., *Ber.*, **16**, 1333–7 (1883).

[13] Foschini, A., and Talenti, M., *Z. anal. Chem.*, **118**, 94–7 (1940).

$$6\,HCHO + 4\,NH_3 \longrightarrow (CH_2)_6N_4 + 6\,H_2O \qquad (4)$$

$$6\,HCHO + 4\,NH_4Cl \longrightarrow (CH_2)_6N_4 + 4\,HCl + 6\,H_2O \qquad (5)$$

and potassium cyanide:[1,3]

$$HCHO + KCN \longrightarrow CH_2(OK)CN \qquad (6)$$

Permanganate,[14,15] bichromate,[16] hydrogen peroxide,[17,18] and silver oxide[19,20] oxidation methods have been described. This last-named technique also was used for the estimation of butyraldehyde.[21] The Cannizarro reaction has been used to some extent:[12,22]

$$2\,HCHO + NaOH \longrightarrow CH_3OH + HCOONa \qquad (7)$$

Gravimetric methods included precipitation with aniline to form the imine,[23] with hydrogen sulfide to form the trithioformaldehyde,[24,25] and with 5,5-dimethyl-1,3-cyclohexanedione (dimedon) to form the methylene bis compound.[26-29] Most of the volumetric and gravimetric methods were reviewed by Craig[30] and Mantegazza.[31] For the determination of acetone, Messinger's iodimetric method[32] has been used widely:[33-39]

$$CH_3COCH_3 + 3\,I_2 + 4\,NaOH \longrightarrow CHI_3 + CH_3COONa + 3\,NaI + 3\,H_2O \qquad (8)$$

---

[14] Smith, H. M., *Analyst*, 21, 148–51 (1896).
[15] Jones, H. C., *Am. Chem. J.*, 17, 539–41 (1895).
[16] Nicloux, M., *Bull. soc. chem.*, (3), 17, 839–40 (1897).
[17] Blank, O., and Finkenbeiner, H., *Ber.*, 31, 2979–81 (1898).
[18] Foschini, A., and Talenti, M., *Z. anal. chem.*, 117, 94–9 (1939).
[19] Orchard, R., *Analyst*, 22, 4 (1897).
[20] Grutzner, A., *Arch. Pharm.*, 234, 634–7 (1896).
[21] Alekse'ev, S. V., and Zvyagina, S. I., *Sintet. Kauchuk*, 5, 19–26 (1936).
[22] Palfray, L., Sabetay, S., and Sontag, D., *Chimie & industrie*, 29, 1037–8 (1933).
[23] Trillat, M. A., *Bull. soc. chim.*, (3), 9, 305–8 (1893).
[24] Baumann, E., *Ber.*, 23, 60–9 (1890).
[25] Hickling, A., and Rodwell, F., *J. Chem. Soc.*, 1941, 51–2.
[26] Vorlander, D., Ible, C., and Volkholz, H., *Z. anal. Chem.*, 77, 321–7 (1929).
[27] Ionescu, M. V., and Bodea, C., *Bull. soc. chim.*, (4), 47, 1408–9 (1930).
[28] Yoe, J. H., and Reid, L. C., *Ind. Eng. Chem., Anal. Ed.*, 13, 238–40 (1941).
[29] Stepp, W., and Feulgen, R., *Z. physiol. Chem.*, 114, 301–6 (1921).
[30] Craig, A. G., *J. Am. Chem. Soc.*, 23, 638–43 (1901).
[31] Mantegazza, A., *Materie plastiche*, 13, 84–6, 117–22 (1935).
[32] Messinger, J., *Ber.*, 21, 3366–72 (1888).
[33] Goodwin, L. F., *J. Am. Chem. Soc.*, 42, 39–45 (1920).
[34] Collischonn, F., *Z. anal. Chem.*, 29, 562–72 (1890).
[35] Squibb, E. R., *J. Am. Chem. Soc.*, 18, 1068–79 (1896).
[36] Kebler, L. F., *Ibid.*, 19, 316–20 (1897).
[37] Keppeler, G., *Z. angew. Chem.*, 18, 464–5 (1905).
[38] Marriot, W. M., *J. Biol. Chem.*, 16, 281–8 (1913).
[39] Haughton, C. O., *Ind. Eng. Chem., Anal. Ed.*, 9, 167–8 (1937).

Probably the most widely applicable procedures for the determination of carbonyl compounds were based on reaction with hydroxylamine hydrochloride:

$$RCHO + H_2NOH{\cdot}HCl \longrightarrow RCH{=}NOH + HCl + H_2O \tag{9}$$

$$RCOR' + H_2NOH{\cdot}HCl \longrightarrow RR'C{=}NOH + HCl + H_2O \tag{10}$$

The acidimetric technique was first applied to formaldehyde[40] and later found applicable to a number of carbonyl compounds.[39,41–48] The method was improved by Bryant and Smith[49] who used pyridine for the double purpose of assisting oxime formation and of rendering the initial reaction mixture neutral to bromophenol blue, thus eliminating the need of a blank titration. Most of the analytical procedures employing this reagent were subject to interference from acidic and basic substances. Also, the permissible concentrations of organic solvents were restricted, particularly in some cases where they affected the indicator.

The reaction of carbonyl compounds with hydroxylamine hydrochloride has been applied analytically in another way. Equations (9) and (10) indicate that 1 mole of water was formed for each mole of carbonyl which had reacted. The water formed could be determined by titration with Karl Fischer reagent.[50] This reagent required no indicator and was not affected by large amounts of inert solvents. Since it did not depend on acidimetry, no interference was encountered from large excesses of acidic materials.

B. Development of the Method

From an examination of the data obtained by Bryant and Smith[49] in their acidimetric hydroxylamine procedure, it was evident that elevated temperatures were necessary to effect complete reaction of many carbonyl compounds. The ease with which cyclohexanone formed the oxime, however, indicated that room temperature might

---

[40] Brochet, A., and Cambier, R., *Compt. rend.*, 120, 449–54 (1895).
[41] Bennett, A. H., *Analyst*, 34, 14–7 (1909).
[42] Bennett, A. H., and Donovan, F. R., *Ibid.*, 47, 146–52 (1922).
[43] Bennett, A. H., and Salamon, M. S., *Ibid.*, 52, 693–5 (1927).
[44] Bennett, A. H., and Cocking, T. T., *Ibid.*, 56, 79–82 (1931).
[45] Marasco, M., *Ind. Eng. Chem.*, 18, 701–2 (1926).
[46] Schultes, H., *Angew. Chem.*, 47, 258–9 (1934).
[47] Leithe, W., *Fette u. Seifen*, 11, 615–6 (1938).
[48] Eitel, A., *J. prakt. Chem.*, 159, 292–303 (1942).
[49] Bryant, W. M. D., and Smith, D. M., *J. Am. Chem. Soc.*, 57, 57–61 (1935).
[50] Mitchell, J., Jr., Smith, D. M., and Bryant, W. M. D., *Ibid.*, 63, 573–4 (1941).

be satisfactory for this compound. In initial studies 10 ml. portions of a methanol solution, having 9.6 millimoles of cyclohexanone, were treated with 40 ml. of 0.5 *N* hydroxylamine hydrochloride in methanol alone or with the same volume of reagent plus an added 3 ml. of pyridine. After 30 minutes to 1 hour at room temperature, the solutions were titrated with Karl Fischer reagent. A fairly sharp endpoint was obtained on the samples but the blanks faded too rapidly to permit an accurate determination of their water contents. It was estimated that the reaction of the cyclohexanone was at least 90 per cent complete in each case, based on the first endpoint obtained on the pyridine-free blank which faded less rapidly than the one containing the base.[51]

Fischer reagent reacted with hydroxylamine salts to indicate apparent water (page 263, Chapter VIII); oximes, on the other hand, were inert toward the reagent.

Obviously the success of the method depended on finding a reagent which would combine with the excess hydroxylammonium ion. The fact that the samples could be titrated fairly successfully indicated that excess water might have some effect. It was found that if sufficient water were added to the blanks the endpoints were as good as those of the samples. At the same time it was remembered that esters tended to react with hydroxylamine to form the hydroxamic acid; for example, ethyl acetate should give acethydroxamic acid:

$$CH_3COOC_2H_5 + H_2NOH \longrightarrow CH_3C(OH){=}NOH + C_2H_5OH \qquad (11)$$

It was found that if excess ethyl acetate were added after the carbonyl compound had reacted, but before titration with Karl Fischer reagent, fairly stable endpoints were obtained. Portions of a methanol solution, each containing 4.8 millimoles of cyclohexanone, were treated with 30 ml. of 0.5 *N* hydroxylamine reagent and 25 ml. of pyridine. After ½ hour at room temperature, 25 ml. of C.P. absolute ethyl acetate were added and the solution was titrated for water. Values of 98.3 ± 0.2 per cent ketone were found, compared to 98.4 per cent by the acidimetric procedure.[49] By heating for 2 hours at 60°C. instead of allowing the samples to stand at room temperature, the authors obtained quantitative results on several other carbonyl compounds, including vanillin, dimethylbenzaldehyde, diisopropyl

---

[51] Unpublished results from the authors' laboratory.

ketone, and benzophenone. The endpoint of the titration was not always permanently stable. It was observed, however, that when an appreciable volume of discharged Karl Fischer reagent was present in hydroxylamine solutions, a perfectly stable endpoint was obtained. Apparently, when sufficient water was present and Fischer reagent was added rapidly, sufficient spent reagent became available to combine in some way with the hydroxylamine. The most likely component of Fischer reagent appeared to be sulfur dioxide, which was known to form sulfamic acid:

$$H_2NOH \cdot HCl + SO_2 + 2\,C_5H_5N \longrightarrow H_2NSO_3H \cdot C_5H_5N + C_5H_5N \cdot HCl \qquad (12)$$

This would be a modification of Raschig's synthesis of sulfamic acid from hydroxylamine and sulfur dioxide.[52] Pyridine was found to accelerate the reaction. Crystals of pyridinium sulfamate (dec. 117°C.) were recovered and identified by comparison of their properties under the petrographic microscope with known optical crystallographic data.

On this assumption, a solution of SO_2 in pyridine was prepared. This reagent was effective, added in excess to hydroxylamine hydrochloride solution, since stable Fischer titration endpoints then were obtained. Sulfur dioxide in methanol apparently was satisfactory except that the reaction with hydroxylamine was considerably slower. With pyridine present the reaction was complete within 10 minutes at room temperature. It was found that a 1 *M* solution of pyridine-sulfur dioxide in methanol was quite easily prepared, was quite stable, and, when added in excess, was completely effective in stabilizing the endpoint of the Karl Fischer reagent titration.[50]

C. Reagents

Approximately 0.5 *N* hydroxylamine hydrochloride solution is prepared by dissolving 35 grams of the dry finely powdered C.P. salt in dry methanol (less than 0.1 per cent water) to make 1 liter of solution. A 1 *M* solution of sulfur dioxide and pyridine in methanol is prepared by adding 45 ml. of liquid sulfur dioxide to a chilled solution of 80 ml. of pyridine and 875 ml. of methanol. (Moisture initially present in the reagents should be kept to a minimum since this water adds to the blank titers. The solutions can be prepared readily in dry methanol.) C.P. pyridine is also required and C.P. methanol may be used as a solvent for samples.

---

[52] Raschig, F., *Ber.*, 20, 584–9 (1887).

D. General Analytical Procedure

Using rapid delivery pipets or automatic graduates* (Fig. 47), 30 ml. of hydroxylamine hydrochloride reagent and 5 ml. of pyridine are transferred to a 250 ml. glass-stoppered volumetric flask. Then the sample, containing up to 10 milliequivalents of carbonyl (no more than 15 milliequivalents of carbonyl plus water), is added. The flasks, including a blank, are placed in a water bath at 60 ± 1°C.; the stoppers are loosened momentarily for the expulsion of included air, are then tightened, and the mixtures heated for 2 hours. At the end of this time the flasks are removed and allowed to cool to room temperature. Twenty-five milliliters of 1 *M* pyridine-sulfur dioxide in methanol are added and, after standing at room temperature for from 10 minutes to 1 hour, the solution is titrated for water with Karl Fischer reagent.

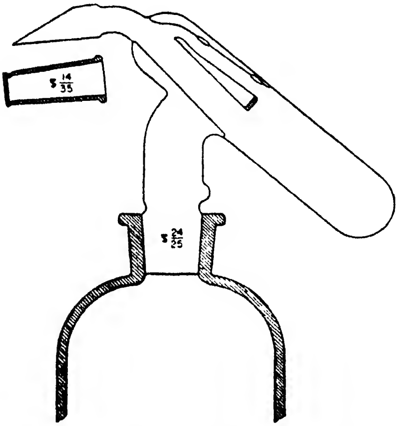


Fig. 47. Automatic graduate.[51]

Ten minutes at room temperature are sufficient for the reaction to go to completion. Crystals of pyridinium sulfamate occasionally appear before or during titration. This compound is without action on the Fischer reagent. The endpoint is matched by observing the supernatant liquid. Precipitation is greater after the mixture has stood for periods longer than 1 hour; since this tends to obscure the endpoint, it is recommended that the titration be made within an hour.

Initial water content of the sample is determined after conversion of the aldehyde or ketone to the cyanohydrin as outlined on pages 23 and 151. (For the more stable carbonyl compounds, the free water may be determined by direct titration of the sample dissolved in excess pyridine; see page 146 ff., Chapter V.)

The titer of the sample less that of the blank is calculated to millimoles of water. From this figure are deducted the millimoles of water originally present in the sample. The net value is equivalent, mole for mole, to the carbonyl present.

---

* The Gribbins "Autograds" were made for the authors by the du Pont Experimental Station glassblowers.

Nonacidic samples often can be dissolved in methanol and a suitable aliquot, for example, 10 ml., taken for analysis. A volume of diluent equal to the aliquot should also be added to the blank. Under these conditions the calculation for net water must be modified to take account of the difference between the amount of solvent in the sample and that in the blank. This is done with sufficient accuracy by correction for the volume of sample in the aliquot. (With dry methanol this difference is usually insignificant, but C.P. pyridine often contains sufficient water to make this correction appreciable.) The order of addition, hydroxylamine reagent and pyridine followed by sample, minimizes any tendency toward esterification when an acidic sample is weighed directly. If necessary, the volume of pyridine used can be increased with acidic materials.

A typical analysis of a 0.8290 gram sample of 2,6-dimethyl-4-heptanone (diisobutyl ketone, M.W. = 142.2) illustrates the calculations involved:

Solution	Fischer reagent, ml. (0.1695 millimole H_2O/ml.)	Water found, millimoles	
		Gross	Net
25 ml. pyridine	10.2	(*a*) 1.73	
4 grams sample + 25 ml. pyridine	11.5	(*b*) 1.95	(*b′*) 0.22 = (*b* − *a*) ≈ 0.1% H_2O
Blank (30 ml. $H_2NOH \cdot HCl$ reagent + 5 ml. pyridine + 25 ml. SO_2 reagent)	7.7	(*c*) 1.30	
Sample solution (30 ml. $H_2NOH \cdot HCl$ reagent + 5 ml. pyridine + sample + 25 ml. SO_2 reagent)	42.2	(*d*) 7.15	(*d′*) 5.85 = (*d* − *c*)

Calculated millimole free water in 0.8290 gram sample (from *b′*) =

$$\frac{0.22 \times 0.8290}{4} = 0.04$$

$$\frac{(5.85 - 0.04) \times 142.2}{1000 \times 0.8290} \times 100 = 99.7\% \text{ diisobutyl ketone}$$

$$99.7\% + 0.1\% = 99.8\% \text{ total}$$

E. Experimental Results

Results obtained with a number of aldehydes and ketones are given in Table CXXI. Except where noted the results by the Fischer reagent procedure are for a heating period of 2 hours at

60°C. and comparative data by the acidimetric method[49] are for a heating period of 2 hours at 100°C. In most cases the C.P. chemicals were used as received.

TABLE CXXI

Analytical Data for Aldehydes and Ketones[50]

Substance	Carbonyl found, wt. %[a]		Water, wt. %	Total, wt. %
	Acidimetric method,[b]	Karl Fischer titration		
Formaldehyde[c]		(4)[d] 37.1 ± 0.2[e]	50.6	87.7
n-Butyraldehyde	93.9 ± 0.5	(2) 94.2 ± 0.2	0.2	94.4
2-Ethylbutyraldehyde	83.2 ± 0.8	(6) 83.5 ± 0.6	0.2	83.7
2-Ethylhexanal	95.8 ± 0.4	(2) 98.3 ± 0.2	0.1	98.4
Dextrose		(4) 102.2 ± 1.0	0.0	102.2
Benzaldehyde	98.6	(4) 98.6 ± 0 1[e]	0.1	98.7
Dimethylbenzaldehyde	98.5 ± 0.5	(4) 98.6 ± 0.2	0.05	98.6
Salicylaldehyde	98.1 ± 0.1	(2) 99.7 ± 0.2	0.1	99.8
Cinnamaldehyde	99 2	(2) 99.1 ± 0.2	0.1	99.2
Vanillin		(2) 99.8 ± 0.2	0.05	99.8
Acetone		(6) 98.9 ± 0.2	0.4	99.3
		(2) 99.6 ± 0.2[e]	0.4	100.0
2-Methyl-3-butanone	83.4 ± 0.4	(4) 83.5 ± 0.3	1.2	84.7
2-Methyl-4-butanone	96.9 ± 0.6	(4) 97.5 ± 0.2	0.1	97.6
2,4-Dimethyl-3-pentanone		(4) 99.5 ± 0.2	0.05	99.5
2,6-Dimethyl-4-heptanone		(10) 99.4 = 0.3	0.1	99.5
Cyclopentanone	97.6 ± 0.2	(6) 98.6 ± 0.1	0.2	98.8
Cyclohexanone	99.0 ± 0.2	(8) 99.0 = 0.3[e]	0.1	99.1
dl-Camphor	98.8[f]	(2) 84.6 ± 0.2	0.0	84.6
		(2) 96 1 ± 0.0[g]	0.0	96.1
Benzophenone	100.9[h]	(2) 100.3 ± 0.0	0.0	100.3
Benzoin	102.4[i]	(3) 102.7 ± 1.2	0.0	102.7

[a] With maximum deviation from the mean.
[b] Acidimetric hydroxylamine procedure.[49]
[c] 37% formalin contained about 12% methanol.
[d] Figures in parentheses represent number of individual determinations.
[e] 0.5 hour at room temperature (27 ± 2°C.)
[f] 5 hours at 100°C.
[g] 4 hours at 60°C.
[h] 3 hours at 100°C.
[i] 1 hour at 100°C.

It was evident from Table CXXI that the method was applicable to aldehydes and ketones of the aliphatic, alicyclic, and aromatic series. Camphor, apparently, was the only substance which failed to react completely. Low total results in some cases were almost certainly due to free acid impurity. The average precision of this procedure was about ±0.3 per cent. Several known mixtures were prepared gravimetrically using materials of known purity.[51] In most cases the samples were analyzed for all components. Typical data are shown in Table CXXII.

TABLE CXXII

Analysis of Synthetic Mixtures[51]

No.	Mixture	Composition, wt. %	
		Calculated	Found[a]
1	Cyclohexanone	10.0	10.0 ± 0.1
	Acetic acid	89.5	89.4
	Water	0.5	0.6 ± 0.0
2	Cyclohexanone	40.6	40.4 ± 0.1
	Acetic acid	58.2	58.2
	Water	1.2	1.2 ± 0.1
3	Cyclohexanone	9.2	9.3 ± 0.1
	Acetic acid	86.5	86.5
	Water	4.3	4.3 ± 0.1
4	Cyclohexanone	11.8	11.7 ± 0.1
	Cyclohexane	87.1	
	Cyclohexanol	1.0	0.9
	Water	0.1	0.1 ± 0.0
5	Cyclohexanone	51.8	51.9 ± 0.3
	Cyclohexanol	47.8	47.5
	Water	0.4	0.2 ± 0.0
6	Acetone	56.2	55.8 ± 0.2
	Methanol	41.0	40.2
	Water	2.8	3.0 ± 0.1
7	Acetone	56.6	56.8 ± 0.2[b]
	Water	43.4	43.5 ± 0.2
8	Butyraldehyde	75.0	74.6 ± 0.2
	Butyric acid	4.6	4.8
	Ethyl acetate	18.5	
	Water	1.9	2.0 ± 0.1

[a] Acetic acid determined by titration with standard alkali; butyric acid, with standard sodium methylate; alcohol, with acetylpyridinium chloride;[53] water, with Karl Fischer reagent after treatment with hydrogen cyanide (page 23, Chapter II). [b] 0.5 hour at room temperature; all others, 2 hours at 60°C.

Samples 3 and 4 of Table CXXII also were analyzed for ketone by allowing the solutions with the general reagents (hydroxylamine hydrochloride solution plus pyridine) to stand for ½ hour at room temperature. Results of 9.2 ± 0.0 and 11.7 ± 0.1 per cent cyclohexanone were found, respectively.

F. Interfering Substances

The general method was applicable without modification to solutions containing high concentrations of acids, ethers, esters, alcohols, or hydrocarbons.

---

[53] Smith, D. M., and Bryant, W. M. D., *J. Am. Chem. Soc.*, 57, 61–5 (1935).

Free alkalies titrated mole for mole as water, so that quantitative corrections could be made readily. In the presence of an esterification catalyst organic acids reacted with the methanol to form water; therefore samples containing acidic compounds, both inorganic and organic, were diluted with pyridine instead of methanol.

Acetal and ketal formations with liberation of water were quite rapid in methanol in the presence of hydrogen ions. A 10 per cent solution of cyclohexanone in methanol containing a trace of hydrochloric acid had reacted to the extent of 50 per cent after 5 days at room temperature, while butyraldehyde and benzaldehyde in the same environment gave 30 to 45 per cent reaction after 24 hours, based on water formed.[51] Pyridine reacted with the acid, which eliminated any tendency toward acetal formation and thereby avoided a correction for the free water determination.

Peroxides interfered, apparently through reaction with the hydroxylamine. A sample of 2.38 millimoles of cyclohexanone containing 0.21 millimoles of benzoyl peroxide was treated according to the general carbonyl procedure. A net average of 3.02 millimoles of apparent water was found, an excess of 0.64 millimole, indicating for this experiment that in effect 3 moles of water were formed per mole of peroxide added.[51]

When titrated directly with Fischer reagent, benzoyl peroxide slowly oxidized the hydrogen iodide always present in the reagent to free iodine (see page 143, Chapter V).

Anhydrides interfered unless subjected to prior hydrolysis. It was originally thought, for example, that acetic anhydride would react with hydroxylamine to form the hydroxamic acid and acetic acid or with methanol to form the ester and acid:

$$(CH_3CO)_2O + HONH_2 \longrightarrow CH_3C(OH){=}NOH + CH_3COOH \qquad (13)$$

or:

$$(CH_3CO)_2O + CH_3OH \longrightarrow CH_3COOCH_3 + CH_3COOH \qquad (14)$$

Since no water was involved in either equation (13) or (14), no interference was expected. However, on direct analysis, a sample containing 11 per cent acetaldehyde and 35 per cent acetic anhydride in acetic acid gave a value of about 20 per cent aldehyde. This interference was eliminated by hydrolysis of the anhydride before addition of the hydroxylamine reagent.[51] Three known mixtures (A, B, and C) were prepared in toluene solution and stored at 0°C. Suitable duplicate aliquots were added to 250 ml. glass-stoppered volumetric flasks containing 25 ml. of 10 per cent sodium iodide plus 1 per cent water in pyridine (see Chapter XI). The solutions were left for 1 hour at room temperature; then 30 ml. of 0.5 *N* hydroxylamine hydrochloride in methanol were added. After an additional ½ hour at room temperature, 25 ml. of pyridine-sulfur dioxide were added and the solutions were titrated with Fischer reagent. Net water* found was calculated as aldehyde, as given in Table CXXIII.

TABLE CXXIII

Determination of Acetaldehyde in Presence of Acetic Anhydride[51]

No.	Mixture	Composition, wt. %	
		Calculated	Found
A	Acetaldehyde Acetic anhydride Acetic acid	10.0 30.3 59.8	10.8 ± 0.4
B	Acetaldehyde Acetic anhydride Acetic acid	24.6 29.8 45.6	25.0 ± 0.5
C	Acetaldehyde Acetic anhydride Ethyl acetate	15.7 45.7 38.6	15.5 ± 0.1

High concentrations of water reduced the sensitivity of the general method; sample size was limited to a maximum of 15 millimoles of carbonyl plus water, because of otherwise unwieldy titers. Thus only 0.27 gram of a 99 per cent water–1 per cent cyclohexanone solution could be used. Duplicate analyses of this solution gave values of 0.3 and 0.7 per cent cyclohexanone. Large concentrations of such amines as did not react with the aldehyde group did not interfere.

Primary amines and aldehydes condense to give the corresponding imine and water (see Chapter XIII). A reaction of this type probably would be complete before the analysis for carbonyl was made and, therefore, actually should not be considered an interfering reaction in the carbonyl method.

G. Other Studies

Using solution 5, Table CXXII, an attempt was made to determine alcohol plus ketone simultaneously. Direct analysis for cy-

---

* Corrected for (water added) minus (water absorbed by anhydride). For unknowns containing anhydrides, a separate determination of the anhydride content would, of course, be required. See page 319, chapter XI.

clohexanol by the general Karl Fischer hydroxyl procedure (Chapter IX) gave a value of 68 per cent as alcohol, indicating about 40 per cent reaction of the ketone, assuming that 1 mole of water was formed per mole of cyclohexanone reacted. A second sample was first treated with the boron trifluoride-in-acetic acid hydroxyl reagent. After 2 hours at 65°C., the solution was allowed to cool to room temperature and 50 ml. of pyridine were carefully added. Then a calculated 100 per cent excess over cyclohexanone present of solid hydroxylamine hydrochloride salt was added. The mixture was allowed to stand for 30 minutes at room temperature, was treated with pyridine-sulfur dioxide in the usual way, and was titrated with Fischer reagent. After correction for the water from esterification of the alcohol, the reaction of the cyclohexanone appeared to be 83–85 per cent complete.[51] It is not known whether this represented incomplete reaction of the ketone or whether a side reaction occurred during esterification.

TABLE CXXIV

Analytical Data for Carbonyl Compounds Reacted at Room Temperature[51]

Substance	Carbonyl found, wt. %		
	Fischer reagent procedure		Acidimetric procedure, 2 hrs., 100°C.
	Without pyridine, 0.5 hr., 27°C.	With pyridine, 2 hrs., 60°C.	
Formaldehyde	37.3 ± 0.1	37.3 ± 0.1[a]	
n-Butyraldehyde	97.3 ± 0.1	97.2 ± 0.2	95.8
Benzaldehyde	95.6 ± 0.2		95.7
Salicylaldehyde	74.4 ± 0.2	98.1 ± 0.2[a]	96.8
Acetone	99.6 ± 0.2	99.6 ± 0.2[a]	
2-Methyl-4-pentanone	96.7 ± 0.5	96.9 ± 0.2	
2-Heptanone	98.3 ± 0.2		98.4
4-Heptanone	90.5 ± 0.3		94.3
Cyclopentanone	76.2 ± 0.1	98.6 ± 0.1	
		92.4 ± 0.3[a]	
Cyclohexanone	91.8 ± 0.2	91.9 ± 0.2	
Acetophenone	74.2 ± 0.0	98.6 ± 0.1[a]	
Benzophenone	12.6 ± 0.3	100.3 ± 0.0	
		25.4 ± 0.1[a]	
Benzoin	54.4 ± 0.5	102.7 ± 1.2	
		88.2 ± 1.2[a]	

[a] 30 minutes at room temperature (27 ± 2°C.).

The value of pyridine as an acid acceptor was apparent[49] but even in its absence many of the lower aldehydes and ketones reacted to completion in 30 minutes at room temperature.[51] Approximately 0.1 *M* quantities of several carbonyl compounds were diluted to 100 ml. with dry methanol. Then milliliter portions were treated with 30 ml. of 0.5 *N* hydroxylamine hydrochloride in methanol reagent. The solutions were allowed to stand for ½ hour at 27 ± 2°C.; then 25 ml. of 1 *M* pyridine-sulfur dioxide in methanol were added. After 10 minutes to 1 hour at room temperature, the mixtures were titrated with Karl Fischer reagent. Results are given in Table CXXIV, together with comparative data by the general Fischer reagent procedure requiring 2 hours at 60°C.,[50] and the acidimetric method.[49]

The milder conditions appeared to be applicable to many aldehydes. Of the four tried only salicylaldehyde failed to react completely in the absence of pyridine. The activity of ketones varied but, in general, the sterically unhindered aliphatic ketones tended to react quantitatively. It was interesting that cyclopentanone reacted only partially, since cyclohexanone reacted with ease.

CHAPTER XIII

Determination of Amines and Nitriles

A. Determination of Primary Plus Secondary Amines

1. Methods of Analysis for Amines

Practical analytical procedures for the quantitative determination of specific classes of organic nitrogen compounds have been limited in number. Amines most often were titrated with standard acid either potentiometrically or visually to an indicator such as methyl red or bromophenol blue in aqueous or alcoholic solution. Weakly basic compounds often could be titrated with perchloric acid in glacial acetic acid solution.[1,2] These procedures ordinarily did not differentiate between types of amines and consequently the values obtained were simply measures of total bases. In some cases the amine hydrochlorides could be recovered and identified.[3]

Ammonia could be removed as the mercury ammonium oxide after treatment with mercuric oxide[4,5] or as hexamethylenetetramine after reaction with formaldehyde.[6,7] Hexamethylenetetramine could be determined by acid hydrolysis, followed by estimation of the formaldehyde.[8]

Acylation techniques have been used to some extent for the identification of primary and secondary amines,[9,10] but little has been reported on corresponding quantitative procedures. By suitable

---

[1] Thomann, J., *Pharm. Acta Helv.*, 5, 11–24 (1930).

[2] Spengler, H., and Kaelin, A., *Hundert Jahre Schweiz. Apoth.-Ver., Centenaire Soc. suisse Pharm., 1843–1943*, pp. 542–64 (1943).

[3] Hierneis, J., *Die Chemie*, 56, 136–8 (1943).

[4] Francois, M., *Compt. rend.*, 144, 567–9, 857–9 (1907).

[5] Pugh, C. E. M., and Quastel, J. H., *Biochem. J.*, 31, 282–5 (1937).

[6] Delepine, M., *Compt. rend.*, 122, 1064–6 (1896).

[7] Benoit, G. J., Jr., and Norris, E. R., *Ind. Eng. Chem., Anal. Ed.*, 14, 823–5 (1942).

[8] Rapine, M., *Ann. chim. anal. chim. appl.*, 25, 113 (1943).

[9] Alexander, J. W., and McElvain, S. M., *J. Am. Chem. Soc.*, 60, 2285–7 (1938).

[10] Billman, J. H., and O'Mahoney, E., *Ibid.*, 61, 2340–1 (1939).

modification of the Smith-Bryant acidimetric acetyl chloride procedure[11] for alcoholic hydroxyl, Olson and Feldman[12] obtained quantitative results on some amines, although in general the reaction varied between 80 and 95 per cent. Under the conditions of their analysis, amides also reacted and consequently this procedure did not differentiate between these classes of compounds. Acetic anhydride has been used indirectly in the determination of tertiary amines by virtue of its preferential reaction with primary and secondary amines. After acetylation, the tertiary amines were determined by titration in glacial acetic acid solution,[13] by distillation of the tertiary amine,[14,15] and by reaction with methyl iodide to form the quaternary compound.[16]

Acetic anhydride also could be used in the determination of primary and secondary amines, according to the reactions:

$$RNH_2 + (CH_3CO)_2O \longrightarrow CH_3CONHR + CH_3COOH \quad (1)$$

$$RNHR' + (CH_3CO)_2O \longrightarrow CH_3CONRR' + CH_3COOH \quad (2)$$

$$(CH_3CO)_2O + H_2O \longrightarrow 2\,CH_3COOH \quad (3)$$

The excess acetic anhydride after reaction was hydrolyzed and the excess water was determined by titration with Karl Fischer reagent under conditions used for the analysis of acetic anhydride (Chapter XI).[17]

This procedure was applicable to a wide variety of primary and secondary amines including aliphatic, alicyclic, heterocyclic, and aromatic types. However, diaryl secondary amines and pyrrole failed to react. Since tertiary amines did not react, the method could be used indirectly for the determination of these substances by correcting the total base titer for primary plus secondary amines.[17]

For the direct determination of tertiary amines, Wagner, Brown, and Peters[17a] made use of the reactions of ammonia and primary and secondary amines with acetic anhydride. After acetylation, the tertiary amines were titrated directly with perchloric acid in acetic acid.[1,2]

---

[11] Smith, D. M., and Bryant, W. M. D., *Ibid.*, **57**, 61–5 (1935).

[12] Olson, V. R., and Feldman, H. B., *Ibid.*, **59**, 2003–5 (1937).

[13] Blumrich, K., and Bandel, G., *Angew. Chem.*, **54**, 374–5 (1941).

[14] Haslam, J., and Guthrie, A. H. S., *Analyst*, **68**, 328–30 (1943).

[15] Haslam, J., and Hearn, P. F., *Ibid.*, **69**, 141–5 (1944).

[16] Wilson, H. N., and Heron, A. E., *Ibid.*, **70**, 38–43 (1945).

[17] Mitchell, J., Jr., Hawkins, W., and Smith, D. M., *J. Am. Chem. Soc.*, **66**, 782–4 (1944).

[17a] Wagner, C. D., Brown, R. H., and Peters, E. D., *Ibid.*, **69**, 2609–10 (1947).

2. DEVELOPMENT OF THE METHOD

Ten milliliter portions of a pyridine solution of butylamine or dibutylamine (10 milliequivalents of amine) were treated with a 100 per cent excess of acetic anhydride in pyridine. Nearly quantitative acetylation was obtained after 30 or 60 minutes at room temperature, as determined by alkaline hydrolysis of the excess anhydride (Chapter XI). When a 30 minute or 1 hour heating period at 60°C. was used, the reaction was complete. When a 200 per cent excess of anhydride was used (either 20 ml. of 1.5 *M* or 5 ml. of 6 *M* acetic anhydride in pyridine), the acetylation was stoichiometric after 30 minutes at room temperature; heating for periods up to 1 hour at 60°C. had no interfering effect on the analysis. The less concentrated (1.5 *M*) reagent, however, proved to be the more stable and therefore was adopted as standard.

3. REAGENTS

The acetylating reagent is prepared by mixing 1.5 moles (142 ml.) of C.P. acetic anhydride in sufficient dry pyridine to make 1 liter of solution.

The acetylating reagent is quite stable and, if kept tightly stoppered, will be effective indefinitely. Some degradation may occur (Wilson and Hughes[18] found that some resinous condensation product was formed when dry pyridine was heated with acetic anhydride), but beyond using up some of the acetic anhydride, the reaction products have no apparent effect on the analysis.

The hydrolysis reagent contains 100 grams of C.P. dry sodium iodide plus water to give 31 to 32 grams total in 1 liter of pyridine solution. (The amount of water to be added to the hydrolysis reagent should be governed by the quantity of water in the pyridine and sodium iodide.) C.P. dry pyridine is used as a sample solvent.

4. ANALYTICAL PROCEDURE

The sample containing up to 10 milliequivalents of primary plus secondary amine is transferred directly or in pyridine solution to a 250 ml. glass-stoppered volumetric flask. Exactly 20 ml. of the acetic anhydride reagent are added. The flasks, including at least one blank, are stoppered and allowed to stand for 30 minutes at room temperature. At the end of this time 25 ml. (calibrated pipet)

---

[18] Wilson, H. N., and Hughes, W. C., *J. Soc. Chem. Ind.*, **58**, 74–7 (1939).

Solution	Fischer reagent, ml. (0.2080 millimole H_2O/ml.)	Water found, millimoles	
		Gross	Net
10 ml. CH_3COOH	7.6	(*a*) 1.58	
10 ml. C_5H_5N	4.8	(*b*) 1.00	(b') 0.83/8.25 ml. C_5H_5N
10 ml. aliquot + 10 ml. CH_3COOH (free water analysis)	12.2	(*c*) 2.54	(c') $0.13 = [c - (a + b')] \Leftrightarrow$ 0.15% H_2O
20 ml. $(CH_3CO)_2O$ reagent + 10 ml. C_5H_5N + 25 ml. NaI–C_5H_5N solution (control) (25 ml. pipet calibration = 0.9993)	25.8	(*d*) 5.36/24.98 ml.	
10 ml. NaI–C_5H_5N solution (hydrolysis reagent (10 ml. pipet calibration = 0.9976)	67.0	(*e*) 13.93/9.976 ml.	
Calculated H_2O/24.98 ml.)		(*f*) 34.81	
10 ml. aliquot + reagents (see (*d*) above) after reaction (sample solution)	73.4	(*g*) 15.27	

Acetic anhydride added:

From (*f*) 34.81 millimoles H_2O in NaI solution
From (*b*) $\underline{1.00}$ millimoles H_2O in 10 ml. C_5H_5N
35.81 millimoles total H_2O added
From (*d*) $\underline{5.36}$ millimoles H_2O
30.45 millimoles $H_2O \Leftrightarrow (CH_3CO)_2O$

Sample added:

From (*f*) 34.81 millimoles H_2O in NaI solution
($b' + c'$) $\underline{0.96}$ millimoles H_2O in 8.25 ml. C_5H_5N + 1.75 ml. amine
35.77 millimoles total H_2O added
From (*g*) $\underline{15.27}$ millimoles H_2O found
20.50 millimoles H_2O used up $\Leftrightarrow (CH_3CO)_2O$ not acetylated

30.45 − 20.50 = 9.95 millimoles $(CH_3CO)_2O$ used up $\Leftrightarrow$ amine present

$$\frac{9.95 \times 129.2}{1000 \times 1.3097} \times 100 = 98.5\text{ \% dibutylamine}$$

$$98.5\% + 0.15\% = 98.65\% \text{ total}$$

This general method of calculation usually is desirable since it permits an evaluation of acetic anhydride reagent strength; see (*e*) and (*f*) above. Once this has been established, however, the analysis can be simplified by assuming the values for (*e*) and (*f*). The net anhydride consumed then may be calculated as the difference between the net water found in the final sample solution (corrected for free water) and the net water found in the control (corrected to the same pyridine content as the sample solution). For example, in this analysis:

20 ml. $(CH_3CO)_2O$ reagent + 8.75 ml. C_5H_5N + 25 ml. NaI–C_5H_5N solution (control)................5.19 millimoles = $(d - b + b')$
10 ml. aliquot + reagents − free water in sample........15.14 millimoles = $(g - c')$
Net $(CH_3CO)_2O$ consumed.........................15.14 − 5.19 = 9.95 millimoles amine

of the hydrolysis reagent are added. The flasks are placed in a water bath at 60 ± 1°C. After being momentarily loosened to allow for expulsion of the expanded air, the stoppers are seated firmly and the flasks are left in the bath for 30 minutes. After cooling spontaneously to room temperature, the solutions are titrated directly for water with Karl Fischer reagent.

The quantity of water added in the hydrolysis solution is determined by titrating a 10 ml. portion (calibrated pipet) with Fischer reagent, and from this calculating the water contained in 25 ml. of reagent.

About 30 millimoles of anhydride are used for the analyses, requiring over 30 millimoles of water to insure complete hydrolysis in the blanks. It is not practical to titrate the large volume of hydrolysis reagent used in an actual analysis. However, it is quite satisfactory to titrate accurately a 10 milliliter portion (containing 13 to 17 millimoles) and from this titer to calculate the exact quantity of water in the 25 ml. of hydrolysis reagent.

Free water originally present is determined by titration of a portion of the sample acidified with glacial acetic acid.

Total anhydride added is equivalent to the net water used up during hydrolysis of the blank. Anhydride not reacted by acetylation is determined by subtracting water found from total water added to the sample, including free water originally present. The net anhydride is equivalent mole for mole to the primary plus secondary amine in the sample.

The following shows the calculation of a typical analysis: 13.0970 grams (17.5 ml.) of dibutylamine (M.W. = 129.16) were diluted to exactly 100 ml. in pyridine (thermostated). A 10 ml. portion contained very nearly 1.75 ml. of amine and 8.25 ml. of pyridine. See table on page 340.

5. Experimental Results

Typical primary amines analyzed by the acetylation method are reported in Table CXXV. These trade products (with the exceptions of hexamethylenediamine, which was sublimed, and *p*-bromoaniline, which was recrystallized from chloroform) were used without further purification. In some cases the purity was checked by acidimetric titration to bromophenol blue.

Analytical data for several secondary amines are given in Table CXXVI. The precisions and accuracies usually were within ±0.2 per cent.

TABLE CXXV

Analytical Data for Primary Amines[17,19]

Amine	Found, wt. %			
	Acidimetric procedure	Acetylation procedure[a]	Water	Total
Butylamine	97.7	(10) 98.0 ± 0.5	1.7	99.7
Propylenediamine		(2) 84.1 ± 0.2	13.9	98.0
Hexamethylenediamine		(4) 99.9 ± 0.1	0.1	100.0
Cyclohexylamine		(2) 100.2 ± 0.2	0.0	100.2
o-Aminodicyclohexyl	96.2[b]	(2) 96.7 ± 0.3	0.0	96.7
Benzylamine	92.8	(2) 92.7 ± 0.2	0.2	92.9
Aniline		(4) 100.8 ± 0.1	0.0	100.8
p-Toluidine		(2) 99.2 ± 0.1	0.0	99.2
p-Phenylenediamine		(4) 99.6 ± 0.1	0.0	99.6
p-Bromoaniline		(4) 99.4 ± 0.1	0.0	99.4
β-Naphthylamine	97.5	(4) 97.1 ± 0.2	0.0	97.1
o-Aminodiphenyl		(2) 99.0 ± 0.2	0.0	99.0
2-Aminopyridine		(2) 100.0 ± 0.3	0.0	100.0
2-Amino-2-methylcyclopentylamine		(4) 96.4 ± 0.2	2.8	99.2
α-Aminoisovaleronitrile		(4) 93.0 ± 0.2	6.2	99.2
γ-Methylthiol-α-aminobutyronitrile		(2) 89.8 ± 0.0	9.8	99.6

[a] Figures in parentheses represent number of individual determinations.
[b] Calculated from Kjeldahl nitrogen value.

TABLE CXXVI

Analytical Data for Secondary Amines[17]

Amine	Found, wt. %			
	Acidimetric procedure	Acetylation procedure[a]	Water	Total
Diethylamine	99.7	(2) 99.7 ± 0.1	0.3	100.0
Dibutylamine	98.3	(10) 98.4 ± 0.2	0.1	98.5
Di-*sec*-butylamine	99.5	(4) 83.5 ± 0.2	0.1	83.6
Diisobutylamine	100.1	(2) 100.1 ± 0.1	0.0	100.1
Methylaniline		(4) 98.8 ± 0.1	0.0	98.8
Morpholine[b]		(2) 104.1 ± 0.2	0.0	104.1
Piperidine	99.4	(2) 99.8 ± 0.2	0.0	99.8
Piperazine hydrate		(2) 45.8 ± 0.2	53.8	99.6

[a] Figures in parentheses represent number of individual determinations.
[b] Practical grade, all others pure grade; all compounds analyzed as received.

The results for the amino nitriles were interesting. Both of the α-amino nitriles studied, $(CH_3)_2CHCH(NH_2)CN$ and $CH_3SCH_2CH_2CH(NH_2)CN$, were acetylated without difficulty.

[19] Unpublished results from the authors' laboratory.

The low result obtained on di-*sec*-butylamine probably indicated the presence of some tertiary amine. Diphenylamine, carbazole, and phenothiazine failed to react, results of 0.0 per cent being obtained in all cases after treatment at room temperature or 1 hour at 60°C. Samples of freshly distilled pyrrole gave values of only 0.6 and 2 per cent at 60°C. after 30 minutes and 3 hours, respectively; at room temperature no reaction occurred. Dicyclohexylamine reacted only slightly; values of 4.9 ± 0.1 per cent were obtained.

6. INTERFERING SUBSTANCES

The conditions of the general method for amines were not sufficiently drastic to effect quantitative acetylation of primary alcohols. (Acetic anhydride has been used widely for the determination of alcoholic hydroxyl; see Chapter IX.) The extent of reaction with *n*-butanol and hydroxyethylethylenediamine was about 86 and 91 per cent, respectively, for the hydroxyl group. By limiting the sample size to a total of 10 milliequivalents of amine plus hydroxyl and including a 30 minute heating period at 60 ± 1°C., after the usual 30 minutes at room temperature, primary aliphatic and apparently phenolic hydroxyl reacted quantitatively, as shown in Table CXXVII. (Compare behavior of nitrogen-free phenols with acetic acid in Chapter IX.)

TABLE CXXVII

Analytical Data for Amino Alcohols[17,19]

Amino alcohol	Found, wt. %		
	Acetylation procedure	Water	Total
Monoethanolamine	99.5 ± 0.1	0.2	99.7
Diethanolamine	99.6 ± 0.1	0.0	99.6
Hydroxyethylethylenediamine	99.1 ± 0.2	0.0	99.1
m-Aminophenol	100.0 ± 0.1	0.0	100.0

With the exception of the substituted phenols, analytical data on other amino primary alcohols could be corrected for hydroxyl by means of the specific boron trifluoride catalyzed esterification procedure reported in Chapter IX.

Amino alcohols containing a secondary hydroxyl group were not completely acetylated under the conditions employed for primary hydroxyl, while those with a tertiary hydroxyl group reacted only slightly. Diisopropanolamine, for example, was esterified to the extent of 80 per cent. By increasing the heating period at 60°C. from 30 minutes to 1 hour, this value was raised to 92 per cent.[17] The effect of the type of alcohol is indicated in Table CXXVIII for which 5 milliequivalents of a butanol were analyzed in the presence and absence of butylamine according to the procedure recommended for primary amino alcohols.

TABLE CXXVIII

Relative Reactivity of Acetic Anhydride with *n*-, *sec*-, and *tert*-Butanol[17]

Substance	Hydroxyl esterfied, %
n-Butanol	100
n-Butanol + *n*-butylamine[a]	100[b]
sec-Butanol	71
sec-Butanol + isobutylamine[a]	72[b]
tert-Butanol	6
tert-Butanol + isobutylamine[a]	13[b]

[a] 5 milliequivalents of amine.
[b] Corrected for quantitative amine reaction.

No interference was encountered from amides, carbamates, nitriles, or tertiary amines. Acetamide, urea, urethan, and acetanilide did not react. Acetonitrile and adiponitrile gave values of 0.7 and 0.4 per cent reaction, respectively, after 30 minutes at room temperature or 1 hour at 60°C. Dimethylaniline reacted to the extent of 1.3 ± 0.1 per cent. This may have been due to secondary amine impurity. Small quantities of benzoyl peroxide had no effect on the procedure. Ten and 20 milligram quantities of this peroxide were added to butylamine, dibutylamine, aniline, and diphenylamine. The first three reacted quantitatively, checking within 0.1 per cent the values obtained in the absence of peroxide. Diphenylamine did not react.

7. OTHER STUDIES

A few experiments indicated that acetyl chloride was not satisfactory as an acetylating agent.[19] Ten milliliter aliquots of amines from pyridine solution were added to acetylpyridinium chloride. The solutions became so dark, probably due to resinification of the pyridine (essentially 10 ml. were present in this case), that visual titrations were impossible.

B. Determination of Primary Amines

1. Methods of Analysis for Primary Amines

Primary amines usually were determined by some modification of Van Slyke's manometric nitrous acid technique:[20]

$$RNH_2 + HNO_2 \longrightarrow N_2 + ROH + H_2O \tag{4}$$

Since secondary amines yielded only the nitrosoamine and water and tertiary amines did not react:

$$R_2NH + HNO_2 \longrightarrow R_2N{\cdot}NO + H_2O \tag{5}$$

the quantity of nitrogen formed served as a measure of primary amine. For small quantities of ethylamine a small azotometer has been used in conjunction with the nitrous acid method.[16] Aromatic amines have been analyzed by titration with nitrous acid.[21] Specific aromatic amines also have been determined by various other methods, such as aniline with picryl chloride[22] or methyl magnesium iodide,[23] and toluidines by diazotization.[24, 25] (Phenylhydrazine has been determined by reaction with iodine[26,27] or iodate.[28]) Occasionally the reactions between primary amines and aldehydes have been used for specific purposes, including reactions with formaldehyde for the identification of aromatic amines[29,30] and preferential combination with the sodium bisulfite–benzaldehyde addition compound for the separation of primary aryl amines from secondary alkylaryl amines.[31] Colorimetric methods have been reported for the identification of primary amines (based on reaction with anhydrobisindandiones[32]) and for monoethanolamine.[33]

---

[20] Van Slyke, D. D., *Proc. Soc. Exptl. Biol. Med.*, 7, 468 (1910).

[21] Phillips, J., and Lowy, A., *Ind. Eng. Chem., Anal. Ed.*, 9, 381–2 (1937).

[22] Spencer, G., and Brumley, J. E., *J. Soc. Chem. Ind.*, 64, 53–5 (1945).

[23] Ritter, F. D., *Ind. Eng. Chem.*, 28, 33–5 (1936).

[24] Evers, H. H., and Strafford, N., *J. Soc. Chem. Ind.*, 46, 114–17 (1927).

[25] Benbrook, C. H., and Kienle, R. H., *Ind. Eng. Chem., Anal. Ed.*, 14, 427–8 (1942).

[26] Fischer, E., *Ber.*, 10, 1331–8 (1887).

[27] von Meyer, E., *J. prakt. Chem.*, 36,.115–9 (1887).

[28] Miller, C. D., and Fierman, N. H., *J. Am. Chem. Soc.*, 59, 161–4 (1937).

[29] Al'per-Yul'chevskaya, B. Ya., *Lab. Prakt. USSR*, 16, 6–7 (1941).

[30] Schoorl, N , *Pharm. Weekblad*, 77, 1381–3 (1940).

[31] Ferry C. W., and Buck, J. S., *J. Am. Chem. Soc.*, 58, 2444–5 (1936).

[32] Wanag, G., *Z. anal. Chem.*, 113, 21–34 (1938.

[33] Shupe, I. S., *J. Assoc. Official Agr. Chem.*, 24, 754–7 (1941).

A widely applicable Fischer reagent procedure has been published,[34] based on the Schiff type reaction:

$$RNH_2 + C_6H_5CHO \longrightarrow C_6H_5CH{=}NR + H_2O \qquad (6)$$

The imine condensation product was inert toward Fischer reagent and the water formed from the rapid quantitative reaction in the presence of pyridine could be titrated with Karl Fischer reagent, after excess aldehyde had been inactivated by means of the cyanohydrin reaction (see Chapter V).

This new method was applicable to aliphatic, alicyclic, and aromatic primary amines including amino alcohols. Amino alcohols which contained a secondary amino nitrogen group also reacted nearly quantitatively. Heterocyclic secondary amines interfered.

When used in conjunction with the Fischer reagent procedure for primary plus secondary amines, the imination technique offered a means for determining rapidly and precisely the primary, secondary and tertiary amine contents of mixtures (if no quaternary bases were present).

For the analysis of mixtures for secondary and tertiary amines, Wagner, Brown, and Peters[34a] combined the primary amines as the imines, according to equation (6) and then titrated with standard acid in an organic environment.

2. Development of the Method

It was observed that 1 milliliter quantities of benzylamine, aniline, and cyclohexylamine when treated with 5 ml. of benzaldehyde reacted appreciably after 15 minutes at room temperature. Combination of the excess aldehyde with hydrogen cyanide in methanol containing sodium cyanide (allowing 30 minutes reaction time at room temperature) permitted an approximate determination of the water formed by titration with Fischer reagent. The endpoints were not stable, however, so that exact results could not be obtained. Substitution of pyridine as solvent for the hydrogen cyanide gave fairly stable endpoints indicating 85 to 90 per cent reaction for the three amines. Apparently because of the insolubility of the sodium cyanide catalyst in this environment an increase to 45 minutes in the time allowed for the cyanohydrin reaction was required to insure a stable endpoint. Once the conditions

---

[34] Hawkins, W., Smith, D. M., and Mitchell, J., Jr., *J. Am. Chem. Soc.*, **66**, 1662–3 (1944).

[34a] Wagner, C. D., Brown, R. H., and Peters, E. D., *Ibid.*, **69**, 2611–4 (1947).

necessary for complete aldehyde combination were established the requirements for stoichiometric imine formation were investigated. Ten milliequivalent portions of benzylamine and butylamine in 10 ml. of pyridine solution were added either to 2 ml. (19.6 millimoles) of distilled benzaldehyde or to 5 ml. of 40 per cent benzaldehyde in pyridine. For both amines the reaction, based on water found, was about 95 per cent complete after 30 minutes at room temperature and 97 to 99 per cent complete after 15 or 30 minutes at 60°C. When 3 ml. of pure benzaldehyde or 5 ml. of 60 per cent benzaldehyde in pyridine were used, the reaction was essentially quantitative in 15 minutes at 60°C. Other aromatic primary amines as well as diamines, however, required an additional 15 minutes at 60°C. to effect complete reaction. Salicylaldehyde or butyraldehyde were not as satisfactory as benzaldehyde for reagents. The former after reaction gave dark solutions which were extremely difficult to titrate; the latter was more inconvenient to handle and was subject to interfering side reactions.[84]

3. Reagents

Freshly distilled benzaldehyde is treated with about 0.1 per cent hydroquinone to minimize oxidation.

A reagent consisting of 40 to 60 per cent benzaldehyde in pyridine may be used, but it is usually more convenient to store and dispense the pure aldehyde. The benzaldehyde should be essentially acid free; as little as 0.1 per cent of hydroquinone is sufficient to inhibit oxidation of the aldehyde.

Dry sodium cyanide is obtained by vacuum desiccation of the c.p. material and is stored in a glass-stoppered bottle. Six per cent hydrogen cyanide is prepared by adding about 85 ml. of liquid hydrogen cyanide to 915 ml. of cold c.p. pyridine. This reagent is prepared and stored most conveniently in a 1 liter glass-stoppered bottle in a well-ventilated hood; c.p. pyridine is also used.

Sodium cyanide is very hygroscopic. If used as received, the salt must be accurately weighed to permit accurate evaluation of its water content. When dried as indicated above, however, the required quantity can be estimated roughly, since only a few thousandths of a per cent of water remain after desiccation.

4. Analytical Procedure

The sample containing up to 0.05 equivalent of primary amine is weighed into a 50 ml. glass-stoppered volumetric flask about one third filled with dry pyridine. After dilution to the mark with more pyridine (thermostated), a 10 ml. portion is transferred to a 250 ml. glass-stoppered volumetric flask.

Where convenient the sample, containing up to 10 milliequivalents of primary amine, can be weighed directly. In this case 10 ml. of pyridine should be added to the reaction mixture.

Three milliliters (29.5 millimoles) of benzaldehyde are added. The flasks, including at least one blank, are placed in a water bath at 60 ± 1°C. After the stoppers are momentarily loosened to permit expulsion of the expanded air, the flasks are restoppered firmly and left for 30 minutes. At the end of this time, they are removed and allowed to cool to room temperature spontaneously. The flasks are transferred to a well-ventilated hood. About 0.2 gram of dry sodium cyanide and 30 ml. of 6 per cent hydrogen cyanide in pyridine are added. The flasks are shaken vigorously for about 1 minute and then set aside in the hood for 45 minutes, after which the mixture is titrated with Karl Fischer reagent to the visual endpoint.

The sodium cyanide catalyst is insoluble in pyridine. Vigorous shaking for a short time is necessary to initiate the cyanohydrin reaction. Because of the extreme toxicity of hydrogen cyanide, the solutions should be kept in a well-ventilated hood even during the titration step. The addition of Fischer reagent should be fairly rapid and the first sharp endpoint taken; occasionally some fading may be observed after the titrated solution has stood for a few minutes.

Solution	Fischer reagent, ml. (0.1411 millimole H_2O/ml.)	Water found, millimoles	
		Gross	Net
10 ml. CH_3COOH	15.1	(*a*) 2.13	
10 ml. C_5H_5N	5.2	(*b*) 0.74	(*b′*) 0.72/9.7 ml. C_5H_5N
10 ml. aliquot + 10 ml. CH_3COOH (free water analysis)	34.6	(*c*) 4.88	(*c′*) $2.03 = [c - (a + b')] \approx 14.1\%$ H_2O
3 ml. C_6H_5CHO + 10 ml. C_5H_5N, etc. (control)	25.5	(*d*) 3.60	(*d′*) $3.58 = (d - b + b')$
10 ml. aliquot + 3 ml. C_6H_5CHO, etc. (sample solution)	82.6	(*e*) 11.66	(*e′*) $6.05 = [e - (d' + c')]$

$$\frac{6.05 \times 74.13}{1000 \times 0.2602 \times 2} \times 100 = 86.1\% \text{ propylenediamine}$$

$$86.1\% + 14.1\% = 100.2\% \text{ total}$$

The water found less that present in the blank and original sample is equivalent to the primary amine. Free water is best obtained by titrating the original sample in acetic acid solution.

Analytical data obtained on the analysis of propylenediamine illustrate the calculations for this procedure: 1.3009 grams of propylenediamine (M.W. = 74.13) were diluted to exactly 50 ml. in pyridine. A 10 ml. aliquot contained 0.2602 gram of diamine and very nearly 9.7 ml. of pyridine. See table on page 348.

5. Experimental Results

Several widely different primary and primary-secondary amines were analyzed by this Fischer reagent method. Results are given in Table CXXIX. With the exception of *p*-bromoaniline, which was recrystallized from chloroform, the trade products were used without further purification. The precision and accuracy usually were within ± 0.2 per cent.

TABLE CXXIX

Analytical Data for Primary Amines[34]

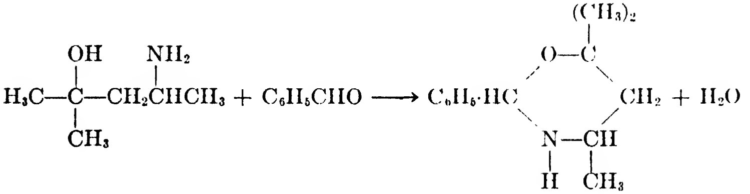
Amine	Analysis for amine by other methods, wt. %	Analysis using Fischer reagent, wt. %		
		Amine[a]	Water	Total
Propylenediamine		(2) 86.3 ± 0.2	14.1	100.4
Butylamine		(6) 98.2 ± 0.2	1.7	99.9
Diethylenetriamine		(4) 96.7 ± 0.1	1.3	98.0
Hexamethylenediamine		(4) 97.2 ± 0.1	2.7	99.9
Decamethylenediamine		(2) 97.0 ± 0.5	1.8	98.8
Laurylamine	98.1[b]	(2) 98.2 ± 0.2	0.6	98.8
Cyclohexylamine	100.2[b]	(4) 99.7 ± 0.2	0.3	100.0
2-Aminomethylcyclopentylamine		(2) 94.2 ± 0.3	5.4	99.6
o-Aminobicyclohexyl	96.7[c,d]	(2) 94.2 ± 0.1	0.1	94.3
Aniline	98.8[c]	(2) 98.8 ± 0.1	0.1	98.9
m-Aminophenol		(2) 99.5 ± 0.1	0.0	99.5
p-Bromoaniline	99.4[c]	(6) 94.8 ± 0.3	0.0	94.8
p-Phenylenediamine	99.6[c]	(4) 99.9 ± 0.0	0.0	99.9
Benzylamine	92.8[b]	(5) 92.5 ± 0.2	0.7	93.2
Toluidine		(2) 97.1 ± 0.3	0.0	97.1
o-Aminobiphenyl	99.0[c]	(2) 92.6 ± 0.2	0.0	92.6
β-Naphthylamine	97.3[c]	(4) 97.2 ± 0.1	0.0	97.2

[a] Figures in parentheses represent number of individual determinations.
[b] By titration to bromophenol blue.
[c] By acetylation procedure for primary plus secondary amines.
[d] 96.2% by Kjeldahl analysis (Table CXXV).

Results with *p*-bromoaniline were precise but consistently about 5 per cent low as compared with the value obtained by the acetylation method. It was not unlikely that aminobicyclohexyl and aminobiphenyl contained some secondary amine.

Amino alcohols containing only primary amine groups reacted quantitatively. Their reaction with benzaldehyde, at least in some cases, might follow one of two courses: either normal imine formation or condensation involving both the amine and hydroxyl groups to form a substituted oxazine type compound.[34]

For example, Kohn[35] isolated 4,6,6-trimethyl-2-phenyltetrahydrometoxazine after treating benzaldehyde with 2-hydroxy-2-methyl-4-pentylamine:



(7)

The exact mechanism for the analytical reaction was not established since, by either route, 1 mole of water would be formed for each mole of amino alcohol present in the sample. Experimental data obtained on several amino alcohols are given in Table CXXX.

TABLE CXXX

Analytical Data for Primary Amino Alcohols[34]

Amino alcohol	Found, wt. %
Monoethanolamine[a]	100.1 ± 0.2
2-Amino-2-methylpropanol	91.1 ± 0.2
2-Amino-2-methyl-1,3-propanediol	91.6
2-Amino-1-butanol	88.9 ± 0.2
Tris-(hydroxymethyl)aminomethane	96.1 ± 0.2

[a] Purified by fractionation, all others technical grade.

Aliphatic and aromatic secondary and tertiary amines did not interfere to any appreciable extent. No reaction was observed with diisobutylamine, methylaniline, diphenylamine, carbazole, triethanolamine, and triisopropanolamine. Values of 1.5 and 2 per cent, respectively, on diethylamine and dibutylamine may have been due to primary amine impurity.[34]

---

[35] Kohn, M., *Monatsh.*, 26, 956–8 (1905).

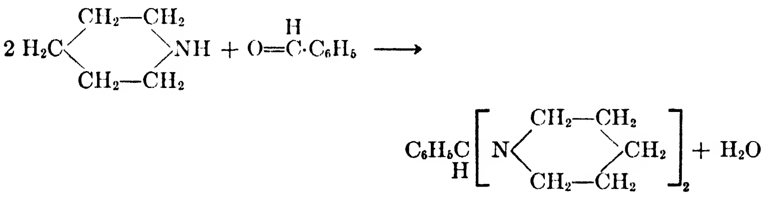
6. Interfering Substances

Amino alcohols containing a secondary amine group usually reacted nearly quantitatively. Following the conditions of the general procedure, diethanolamine gave 1 mole of water per mole amine; diisopropanolamine and *N*-hydroxyethylethylenediamine ($HOC_2H_4NHC_2H_4NH_2$) gave 0.9 and 1.8 moles of water, respectively. A compound boiling at 160°C. (10 mm.) was isolated after reaction of diethanolamine with benzaldehyde. Elemental analyses indicated the compound $C_{11}H_{15}O_2N$:

$$C_6H_5CHO + (HOC_2H_4)_2NH \longrightarrow C_{11}H_{15}O_2N + H_2O \qquad (8)$$

The molecular weight found was 180 as compared to 193 calculated. Both tertiary amino nitrogen and some free hydroxyl* in the unknown suggested that possibly substances containing secondary amino and primary hydroxyl groups may also condense with benzaldehyde to form heterocyclic ring compounds.[34]

Heterocyclic amines reacted with benzaldehyde to form the *N,N'*-benzaldiimines, eliminating 1 mole of water for every 2 moles of amine.[34] For example, with piperidine the reaction was:



(9)

N,N'-Benzaldipiperidine, which melts at 83°C., was isolated as the product from the reaction of 1 mole of piperidine with about 3 moles of benzaldehyde. Literature values for the melting point of this compound varied from 78° to 81°C.[36–38] In addition to piperidine, piperazine and morpholine were reacted according to the standard procedure. Analytical data for the three heterocyclic amines were,

---

* According to equation (7) only one hydroxyl group would be expected to react.

[36] Laun, W., *Ber.*, 17, 675–9 (1884).
[37] Ehrenberg, A., *J. prakt. Chem.*, 36, (2), 117–31 (1887).
[38] Lachowicz, B., *Monatsh.*, 9, 695–700 (1888).

respectively, 0.91, 0.95, and 1.25 moles of water for 2 moles of amine.

No interference was encountered from most amides. Urea and methylurea, however, reacted to the extent of about 10 per cent on the basis of 1 mole of water formed per mole of the urea. If the disubstitution products were formed:[39]

$$2\,H_2NCONH_2 + C_6H_5CHO \longrightarrow C_6H_5{\cdot}CH(NHCONH_2)_2 + H_2O \qquad (10)$$

this value would be doubled since only 1 mole of water would be formed for 2 moles of urea.

Organic acids interfered to some extent; the exact nature of the interference was not determined but may have been due to amine salt formation. Benzaldehyde, containing 10 per cent benzoic acid (about 3 millimoles in the quantity used for analysis) gave only 95 per cent reaction with benzylamine. When alcohols as well as acids were present high results were obtained, possibly because of slight esterification or acetal formation. Thus, when 10 ml. of methanol were present with the benzaldehyde containing 10 per cent acid, values of about 110 per cent were obtained on benzylamine. When acid-free benzaldehyde was used, no interference with methanol was encountered.

C. Determination of Nitriles

1. Methods of Analysis for Nitriles

Nitriles usually were determined by acid or alkaline hydrolysis to the corresponding acids.[40–43] In specific cases alcoholysis[44] or reduction[45,46] has been used. When other nitrogen-containing materials were absent the Kjeldahl technique was applicable.[47,48] Crystalline derivatives, suitable for identification purposes, have

---

[39] Schiff, H., *Ann.*, 291, 367–77 (1896).

[40] Frankland, E., and Kolbe, H., *Ibid.*, 65, 288–304 (1848).

[41] Angelescu, E., Vasiliu, G., and Radvan, S., *Bull. sect. sci. acad. roumaine*, 22, 220–9 (1939).

[42] Rovira, S., and Palfray, L., *Compt. rend.*, 210, 396–8 (1940).

[43] Rabinovitch, B. S., and Winkler, C. A., *Can. J. Research*, 20B, 221–30 (1942).

[44] Backunts, H., and Otto, R., *Ber.*, 9, 1590–1 (1876).

[45] Ladenberg, A., *Ibid.*, 18, 2956–61 (1885).

[46] Cutter, H. B., and Tarras, M., *Ind. Eng. Chem., Anal. Ed.*, 13, 830 (1941).

[47] Rose, E. L., and Ziliotto, H., *Ibid.*, 17, 211–2 (1945).

[48] Petersen, G. W., and Radke, H. H., *Ibid.*, 16, 63–4 (1944).

been prepared by reaction with mercaptoacetic acid[49] and phloroglucinol.[50] These methods were designed primarily for the qualitative analysis of nitriles and did not prove generally applicable to their quantitative determination.

A specific Karl Fischer procedure has been published[51] based on the selective hydrolysis of the nitrile to the corresponding amide:

$$RCN + H_2O \longrightarrow RCONH_2 \qquad (11)$$

The nitrile was treated with an excess of water in the presence of an acid catalyst, $BF_3 \cdot 2CH_3COOH$. After reaction, the unused water was determined by titration with Karl Fischer reagent. Water consumed served as a direct measure of nitrile originally present in the sample. This quantitative procedure was applicable to the determination of aliphatic and most aromatic nitriles.

2. Development of the Method

It was observed in the study of interfering materials in the boron trifluoride catalyzed esterification of alcohols (Chapter IX) that the presence of nitriles led to low results because of their reactive absorption of water. Five millimoles of acetonitrile dissolved in 5 ml. of dioxane reacted to the extent of 4.6 per cent (assuming 1 mole of water absorbed per mole of nitrile) when heated for 2 hours at 65°C. with 20 ml. of acetic acid containing 2 grams of boron trifluoride and 0.27 gram (15 millimoles) of water.[19] When acetic acid was substituted for dioxane as solvent for the nitrile, this value was increased to 9 per cent. On the basis of these observations the possibilities of employing acid hydrolysis as a means of analyzing nitriles were studied. To 5 ml. portions of a dioxane or acetic acid solution containing about 10 milliequivalents of a nitrile were added 20 ml. of boron trifluoride in acetic acid solution containing about 15 millimoles of water. The effects of time, temperature, and concentration of boron trifluoride on the hydrolysis of acetonitrile and adiponitrile are given in Table CXXXI.

Under the conditions of the method the nitriles evidently were hydrolyzed to the corresponding amides much more readily than

---

[49] Condo, F. E., Hinkel, E. T., Fassero, A., and Shriner, R. L., *J. Am. Chem. Soc.*, **59**, 230–2 (1937).

[50] Howells, H. P., and Little, J. G., *Ibid.*, **54**, 2451–3 (1932).

[51] Mitchell, J., Jr., and Hawkins, W., *Ibid.*, **67**, 777–8 (1945).

the amides were hydrolyzed to the acids. Under the most drastic conditions recorded in Table CXXXI ($BF_3 \cdot 2CH_2COOH$, 2 hours at 80°C.), acetamide was hydrolyzed to the extent of 0.3 per cent.

TABLE CXXXI

Factors Influencing Acid Hydrolysis of Nitriles[19,51]

Nitrile	Solvent	Reagent, g. BF_3/l.	Conditions[a]	Reaction,[b] wt. %
Acetonitrile	Dioxane	100	1 hr., R.T.[c]	0.0
		100	1 hr., 60°C.	0.1
		100	2 hrs., 60°C.	4.0
	Acetic acid	100	1 hr., R.T.[c]	0.8
		100	1 hr., 60°C.	3.0
		100	2 hrs., 60°C.	8.5
	Dioxane	360[d]	2 hrs., 60°C.	82
		360	2 hrs., 80°C.	90
	Acetic acid	360	1 hr., 60°C.	66
		360	2 hrs., 60°C.	91
		360	1 hr., 80°C.	95
		360	2 hrs., 80°C.	100
Adiponitrile	Dioxane	100	2 hrs., 60°C.	4.5
		100	2 hrs., 80°C.	27
		360	2 hrs., 80°C.	92
	Acetic acid	100	2 hrs., 60°C.	8.5
		360	1 hr., 60°C.	74
		360	2 hrs., 60°C.	92
		360	1 hr., 80°C.	97
		360	2 hrs., 80°C.	100

[a] 80°C. represented maximum practical temperature without significant loss of water.

[b] Calculated on basis of 1 mole of water used up per mole of nitrile.

[c] Room temperature.

[d] $BF_3 \cdot 2CH_3COOH$.

The conditions for the quantitative hydrolysis of acetonitrile and adiponitrile were found to be applicable to the determination of nitriles in general.

3. REAGENTS

The reagent for the hydrolysis is prepared by adding 6.5 ml. (0.36 mole) of water to 500 ml. of $BF_3 \cdot 2CH_3COOH$. Usually sufficient water is originally present to insure a final concentration of about 0.375 mole per 500 ml. of solution. (The moisture added insures at least a 50 per cent excess of water over nitrile.) Directions for the preparation of $BF_3 \cdot 2CH_3COOH$ were given in Chapter IX. Other reagents include glacial acetic acid and C.P. pyridine.

4. ANALYTICAL PROCEDURE

The sample, containing up to 10 milliequivalents of nitrile, is weighed into a 250 ml. glass-stoppered volumetric flask and 20 ml. of the acid reagent added.

When desirable the sample may be dissolved first in glacial acetic acid and a 5 ml. aliquot used for analysis. Since in this case the acetic acid and a simple aliquot must be titrated separately for water content of the sample, it usually is not necessary to add an equal quantity to the blank. If a water bath is used, the stoppers of the 250 ml. flasks should be loosened momentarily to allow for expansion of included air and then tightened. If an oven is employed, the stoppers must be tightened initially and preferably fixed with a spring clamp.

The flask is stoppered and, together with duplicate blanks, placed in an oven or water bath at 80 ± 2°C. (The temperature should not be permitted to rise above 82°C., for at higher temperatures noticeable quantities of water are lost.) Unless the stoppers are securely sealed, they are momentarily loosened to release expanded air and are resealed. The flasks are heated for 2 hours. At the end of this time, the flasks are removed and allowed to cool to room temperature spontaneously; then they are placed in a container of finely chopped ice during the careful addition to each 15 ml. of C.P. pyridine.

The addition of pyridine just before titration is essential. It combines with the BF_3, thus preventing esterification of the acetic acid with the methanol of the Karl Fischer reagent. The reaction with pyridine is strongly exothermic and is best controlled by adding the pyridine from a pipet, using a circular motion of the pipet tip around the neck of the chilled flask.

The homogeneous solutions are titrated directly with Karl Fischer reagent.

The water remaining in the reacted sample is subtracted from the sum of the water present in the blank plus the free water added with the sample; the net millimoles are equivalent to the nitrile content of the sample. Since the nitrile group does not interfere, the sample can be titrated directly for free water.

The calculations are illustrated in the following typical analysis: 3.0432 grams of butyronitrile (M.W. = 69.10) were diluted to exactly 25 ml. in glacial acetic acid (thermostated). A 5 ml. portion was calculated to contain 0.6086 gram (0.5 ml.) of nitrile and approximately 4.5 ml. of acetic acid.

Solution	Fischer reagent, ml. (0.1667 millimole/ml.)	Water found, millimoles	
		Gross	Net
5 ml. acetic acid	3.5	(*a*) 0.58	(*a'*) 0.53/4.5 ml.
5 ml. aliquot (free water analysis	5.5	(*b*) 0.92	(*b'*) 0.39 = $(b - a')$ $\Bumpeq$ 1.2% H_2O
20 ml. catalyst (control)	100.6	(*c*) 16.79	
5 ml. aliquot + 20 ml. catalyst	53.9	(*d*) 9.88	(*d'*) 8.73 = $(c + b - d)$

$$\frac{8.73 \times 69.10}{1000 \times 0.6086} \times 100 = 99.0\% \text{ butyronitrile}$$

$$99.0\% + 1.2\% = 100.2\% \text{ total}$$

5. Experimental Results

A variety of aliphatic and aromatic nitriles have been analyzed by this method. Those compounds which apparently were hydrolyzed quantitatively to the amides are given in Table CXXXII. In general the precision and accuracy are within ±0.3 per cent.

TABLE CXXXII

Analytical Data for Nitriles[19,51]

Nitrile	Found, wt. %		
	Nitrile[a]	Water	Total
Acetonitrile	(10) 97.6 ± 0.2	2.3	99.9
Propionitrile	(6) 98.7 ± 0.3	1.6	100.3
Cyanoacetamide[c]	(2) 94.7 ± 0.2	0.8	95.5
n-Butyronitrile	(2) 99.0 ± 0.2	1.2	100.2
n-Valeronitrile	(2) 99.4 ± 0.0	0.4	99.8
Trimethylacetonitrile[b]	(2) 100.6 ± 0.1	0.0	100.6
Lauronitrile[b]	(2) 100.2 ± 0.0	0.0	100.2
1,4-Dicyano-2-butene[b]	(4) 99.9 ± 0.0	0.1	100.0
Adiponitrile[b]	(10) 99.8 ± 0.2	0.0	99.8
Sebaconitrile[b]	(4) 100.0 ± 0.4	0.0	100.0
Phenylacetonitrile	(2) 99.6 ± 0.2	0.2	99.8
m-Toluonitrile	(2) 100.6 ± 0.2	0.1	100.7
p-Toluonitrile	(2) 100.8 ± 0.4	0.1	100.9
p-Chlorobenzonitrile	(2) 97.6 ± 0.3	0.1	97.7
β-Naphthonitrile	(6) 96.5 ± 0.4	0.1	96.6

[a] Figures in parentheses represent number of individual determinations.
[b] Purified by fractional distillation; all others analyzed as received.
[c] Practical grade; all others pure grade.

No reference standard method was available for the quantitative determination of nitriles in the presence of other nitrogen-containing compounds. The number of nitriles in Table CXXXII for which essentially a 100% balance was obtained, particularly the five purified nitriles, appeared sufficient to establish the quantitative nature of the method for a wide variety of nitriles. On the possibility that those nitriles on which low totals were obtained might contain nitrogen-free inerts, analyses for total nitrogen were made by the Kjeldahl method using mercuric oxide as catalyst. Cyanoacetamide, chlorobenzonitrile, and naphthonitrile gave values of 95.8, 98.5, and 96.8 per cent, respectively, when calculated from the total nitrogen content. Acetonitrile and adiponitrile also were analyzed by the Kjeldahl procedure giving results of 97.5 ± 0.1 and 99.9 ± 0.1 per cent, calculated as the respective nitriles.[19] An analysis of 1,4-dicyano-2-butene for unsaturation, by a mercury catalyzed bromide–bromate procedure, gave an iodine number of 240 ± 3 compared to a calculated 240.[19] Analysis of this substance by the Kjeldahl method gave 26.35 ± 0.06 per cent compared to a calculated 26.40 per cent total nitrogen.

Some aromatic nitriles apparently did not react completely. Although *m*- and *p*-toluonitrile reacted quantitatively, *o*-toluonitrile and *a*-naphthonitrile gave values of 89.5 ± 0.5 and 80.9 ± 0.2 per cent, respectively.[51] (Based on Kjeldahl values, they analyzed 97.4 and 95.8 per cent, respectively.) The aliphatic dinitrile, cyanoacetonitrile, gave values of 88.8 ± 0.9 per cent and, surprisingly, a practical grade of cyanoacetic acid reacted only to the extent of 37.1 ± 1.4 per cent.[51] (Cyanoacetonitrile analyzed 96.7 per cent by Kjeldahl and cyanoacetic acid, 93.6 per cent.)

Little or no interference was observed with amides. Formamide, acetamide, and adipamide gave values of 0.0, 0.3, and 0.2 per cent, respectively.[51]

This hydrolysis procedure is applicable to many complex organic mixtures. A few typical cases to which the method has been applied are summarized in Table CXXXIII.

6. INTERFERING SUBSTANCES

Materials which interfered with the Karl Fischer hydroxyl procedure (Chapter IX) also affected unfavorably this hydrolysis method for nitriles. Alcoholic hydroxyl was esterified quantitatively, eliminating an equivalent amount of water. Consequently, provided that the hydroxyl content could be determined by an inde-

TABLE CXXXIII

Determination of Nitriles in Presence of Other Nitrogen Compounds and Acids[19]

Known compositions

Substance	Weight per cent			
	Added	Found	Added	Found
Acetonitrile	39.6	39.4 ± 0.1	4.3	4.3 ± 0.1
Acetic acid	40.2		65.7	
Acetamide	13.5		20.6	
Water	6.7	6.7 ± 0.0	9.4	9.3 ± 0.1
Total	100.0		100.0	
Adiponitrile	27.5	27.3 ± 0.2	68.7	68.8 ± 0.2
Adipamide	54.6		13.2	
Adipic acid	13.9		14.8	
Water	4.0	4.1 ± 0.0	3.3	3.3 ± 0.1
Total	100.0		100.0	

Unknown compositions

Substance	Weight per cent found		
Adiponitrile	3.6 ± 0.1[c]	0.2 ± 0.1[c]	13.2 ± 0.2[c]
δ-Cyanovaleramide[a]	27.6 ± 0.2	55.2 ± 0.2	60.1 ± 0.3
Adipamide[b]	67.1	43.3	27.5
Water	1.6	0.5	0.0
Total	99.9	99.2	100.8
Adiponitrile	37.1 ± 0.3	47.6 ± 0.2	
Adipamide[a]	51.5	42.6	
Water	0.7	2.2	
Adipic acid	10.3	3.3	
Ammonium adipate	0.0	3.8	
Total	99.6	99.5	

[a] By acidimetric dehydration method[52] (see page 400, Chapter XVI).

[b] Adipamide separated from the mixture by the use of methylene chloride, only adipamide insoluble.

[c] Analysis corrected for cyanovaleramide content.

pendent method, accurate corrections could be applied to the nitrile value. (In the absence of amino nitrogen the acidimetric acetyl chloride procedure of Smith and Bryant[11] could be used.)

Cyanohydrins, because of the simultaneous hydrolysis of the nitrile group and esterification of the hydroxyl, gave net titers of zero.[19] This was demonstrated on purified cyanohydrins of the following aldehydes: formaldehyde, $CH_2(OH)CN$; acetaldehyde, $CH_3CH(OH)CN$; and β-methylthiolpropionaldehyde, CH_3SCH_2-$CH_2CH(OH)CN$. The presence of amino groups in the same mole-

---

[52] Mitchell, J., Jr., and Ashby, C. E., *J. Am. Chem. Soc.*, **67**, 161–4 (1945).

cule led to low results, possibly at least in part because of their combination with and possible inhibiting influence on the catalytic action of boron trifluoride (see page 292). Aminoacetonitrile,

For example, pyridine when added to the hydroxyl reagent (BF_3 in CH_3COOH) prevented esterification of the acetic acid with the methanol of Fischer reagent during the early stages of the titration.

H_2NCH_2CN, analyzed 95.5 ± 1.5 per cent; methyleneaminoacetonitrile, $CH_2{=}NCH_2CN$, 80.9 ± 0.2 per cent; and α-aminocapronitrile, $H_2N(CH_2)_5CN$, 82.5 ± 0.0 per cent.* γ-Methylthiol-α-aminobutyronitrile analyzed 85.7 ± 0.3 per cent and 9.8 per cent water compared to a value of 89.8 per cent by acetylation of the amino group, according to the procedure for primary plus secondary amines (page 337).

---

* Compare with the other compounds which reacted incompletely (page 357).

CHAPTER XIV

Determination of Peroxides

A. General

In the discussion of the determination of water in the presence of peroxides (page 142 ff., Chapter V), it was pointed out that the reactions of these compounds with Karl Fischer reagent varied according to their structures. Three main classes were considered: (*1*) hydroperoxides (ROOH) and hydrogen peroxide addition compounds ($P \cdot H_2O_2$); (*2*) dialkyl peroxides (ROOR′); and (*3*) diacyl peroxides (RCOOOOCR′).

When titrated with Karl Fischer reagent the peroxides of the first group reacted preferentially with the sulfur dioxide of the reagent to form the acid sulfate (see page 142, Chapter V). Those of the second group, the dialkyl peroxides, were inert toward Fischer reagent, while those of the last group oxidized the hydriodic acid of spent reagent to free iodine (see page 143, Chapter V).

B. Determination of Hydroperoxides and Dialkyl Peroxides

1. METHODS OF ANALYSIS

The dialkyl peroxides were relatively stable; they could not be determined by the commonly employed hydriodic acid reduction techniques. By modifying their iodide reagent to include a ferrous ion catalyst, Wieland and Franke[1] obtained quantitative results on diethyl peroxide. This has been verified in the authors' laboratory.[2] Di-*tert*-butyl peroxide, however, reacted only slightly even under these conditions.[2]

Hydroperoxides, on the other hand, readily oxidized hydriodic acid in the absence of sulfur dioxide:

$$\mathrm{ROOH} + 2\,\mathrm{HI} \longrightarrow \mathrm{ROH} + \mathrm{I_2} + \mathrm{H_2O} \qquad (1)$$

---

[1] Wieland, H., and Franke, W., *Ann.*, **469**, 257–308 (1929).

[2] Unpublished results from the authors' laboratory.

An apparently specific manometric method for the estimation of hydroperoxides was reported by Criegee, Pilz, and Flygare,[3] who measured the oxygen formed from the reaction:

$$\mathrm{RCH_2OOH + Pb(OOCCH_3)_4 \longrightarrow Pb(OOCCH_3)_2 + 0.5\,O_2 + RCHO + 2\,CH_3COOH} \qquad (2)$$

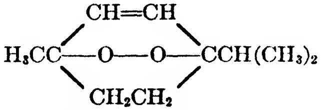
Another method for the determination of diethyl peroxide was reported by Baeyer and Villiger.[4] In their procedure, the peroxide was reduced to the alcohol as a result of the action of zinc on acetic acid or on acetic plus hydrochloric acids, the hydrogen consumption serving as a measure of active oxygen. This reduction technique has been made the basis of a new procedure for dialkyl and hydroperoxides employing Karl Fischer reagent,[2] based on the reactions:

$$\mathrm{ROOR + 2[H] \longrightarrow 2\,ROH} \qquad (3a)$$

$$\mathrm{ROOH + 2[H] \longrightarrow ROH + H_2O} \qquad (3b)$$

$$\mathrm{ROH + CH_3COOH \xrightarrow{BF_3} CH_3COOR + H_2O} \qquad (4)$$

The sample to be analyzed was treated with a reagent consisting of zinc dispersed in boron trifluoride–acetic acid. By simultaneous reduction-esterification, according to equations (3a), (3b), and (4), 2 moles of water are formed for each mole of peroxide reacted.* The water was determined by titration with Karl Fischer reagent. This procedure was applied successfully to the estimation of diethyl and di-*tert*-butyl peroxides and *tert*-butyl hydroperoxide; it probably was applicable to all dialkyl peroxides, except those whose structures involved chemical or steric hindrances. For example, ascaridole:



in effect a dialkyl peroxide, appeared to be unreactive by this procedure.

2. Development of the Method

In initial studies, a sample of diethyl peroxide was reduced first with zinc in acetic acid and then esterified with the boron trifluoride

---

*Equation (4) is the general quantitative reaction for alcoholic hydroxyl (Chapter IX).

[3] Criegee, R., Pilz, H., and Flygare, H., *Ber.*, 72, 1799–1804 (1939).

[4] Baeyer, A., and Villiger, V., *Ibid.*, 33, 3387–93 (1900).

in acetic acid reagent (100 grams of boron trifluoride per liter). It was found that these two reactions could be carried out simultaneously by combining the reagents. High results were observed when the mixture was titrated directly with Fischer reagent. Apparently the reducing environment (unreacted zinc was always present) was sufficient to effect reduction of the iodine of the reagent. This difficulty was eliminated by diluting the mixture with dioxane and titrating a portion of the clear supernatant liquid. (The effect of the small quantity of zinc on the total volume was negligible.)

3. Reagents

The acid reagent, containing about 100 grams of boron trifluoride per liter of solution, is prepared by diluting 210 ml. (278 grams) of $BF_3 \cdot 2CH_3COOH$ to 1 liter with glacial acetic acid. (Details of the preparation of $BF_3 \cdot 2CH_3COOH$ are given in Chapter IX.) C.P. powdered zinc, glacial acetic acid, and C.P. pyridine are used as received; purified dioxane is required. (See page 153.)

4. Analytical Procedure

About 0.3 gram of C.P. powdered zinc is weighed into a dry 100 ml. volumetric flask. Then the sample, containing up to 5 milliequivalents of peroxide, is added, followed by 5 or 10 ml. of acetic acid and 20 ml. of boron trifluoride–acetic acid catalyst solution.

If more convenient, the sample may be dissolved in acetic acid and a 5 or 10 ml. aliquot used for the analysis. In this case the additional acetic acid is unnecessary.

The flask is connected immediately to a desiccant-protected reflux condenser (Fig. 48) with the flask placed in a water bath at 70 ± 5°C. After 2 hours at this temperature, the water bath is removed and the mixture is allowed to cool to room temperature spontaneously. Five milliliters of C.P. pyridine are added carefully through the condenser.

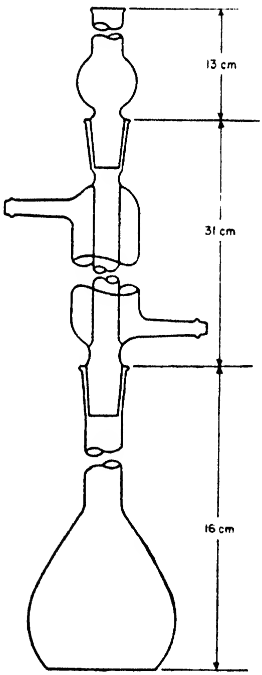


Fig. 48. Apparatus for determination of dialkyl and hydro-peroxides.[2]

The addition of pyridine is necessary to combine with the boron trifluoride; this prevents the interfering esterification reaction of acetic acid with the methanol of Karl Fischer reagent (see Chapter IX).

This is followed by 5 ml. of dry dioxane. When drainage is completed the flask is removed and the contents are diluted to exactly 100 ml. with more dioxane, are shaken, and allowed to settle. A 50 ml. aliquot is titrated with Karl Fischer reagent. At least one blank is run with each set of samples. The net increase in water found, after correction for original free water in the sample, is equivalent to the peroxide. Original free water is determined by direct titration of the sample dissolved in an inert solvent, such as methanol.

A typical analysis of diethyl peroxide (M.W. = 90) illustrates the calculations involved. This sample analyzed 76.0 per cent peroxide by ferrous ion catalyzed hydriodic acid oxidation[1] and 11.0 per cent ethanol by the general Karl Fischer esterification method (Chapter IX). Based on infrared absorption spectra, an appreciable quantity of ethyl ether also was found to be present. A 0.1849 gram sample was weighed into the reaction flask and 10 ml. of glacial acetic acid were added, followed by the reagents. After reaction and dilution to exactly 100 ml. with dry dioxane, a 50 ml. portion was titrated with Fischer reagent (net sample weight was 0.0925 gram). The blank, containing all but the sample, was treated in the same way. Free water was determined on a separately weighed 3.065 gram sample dissolved in 25 ml. of dry methanol.

Solution	Fischer reagent, ml. (0.1590 millimole H_2O/ml.)	Water found, millimoles	
		Gross	Net
25 ml. methanol	1.8	(*a*) 0.29	
Sample + 25 ml. methanol	21.3	(*b*) 3.39	(*b*′) 3.10 = (*b* − *a*) ≈ 1.8% H_2O
50 ml. dioxane solution from blank	23.8	(*c*) 3.78	
50 ml. dioxane solution from sample	35.7	(*d*) 5.68	(*d*′) 1.90 = (*d* − *c*)

Calculated millimole free water in 0.0925 gram sample = 0.09
Calculated millimole free ethanol in 0.0925 gram sample = 0.24

$$\frac{[1.90 - (0.09 + 0.24)] \times 90}{1000 \times 0.0925 \times 2} \times 100 = 76.4\% \text{ diethyl peroxide}$$

5. Experimental Results

Two dialkyl peroxides and one hydroperoxide have been analyzed by this procedure, using 0.3 to 1.65 millimoles of the peroxides. Results are given in Table CXXXIV. The diethyl peroxide results were corrected for 11.0 per cent ethanol (Chapter IX) and 1.3 per cent water found by analysis; *tert*-dibutyl hydroperoxide was corrected for 45.9 per cent *tert*-butanol and 3.5 per cent water. To the authors' knowledge no other reliable method was available for the determination of di-*tert*-butyl peroxide. The highest average value obtained, 95.6 per cent, was found on the commercial material analyzed as received. Other dilutions were calculated on the basis of this figure.

TABLE CXXXIV

Analytical Data for Dialkyl and Hydroperoxides[2]

Peroxide	Solvent	Solution, ml.	Weight per cent	
			Calculated	Found[c]
Diethyl	Acetic acid	10	75.1[a]	(6) 75.6 ± 0.4
	Benzene	5	5.4[a]	(2) 5.4 ± 0.1
	Benzene	25	1.0[a]	(2) 0.9 ± 0.0
Di-*tert*-butyl	Acetic acid	10		(3) 95.6 ± 0.3
	Benzene	5	45.0[b]	(2) 44.4 ± 0.1
	Benzene	50	0.30[b]	(2) 0.24 ± 0.01
tert-Butyl hydroperoxide	Acetic acid	5	50.6[d]	(3) 50.0 ± 0.4

[a] Weight per cent found by ferrous ion catalyzed method.[1]
[b] Calculated value of sample dissolved in benzene.
[c] Figures in parentheses represent number of individual determinations.
[d] Weight per cent found by hydriodic acid oxidation; see Chapter V.

Diacyl peroxides, as exemplified by benzoyl peroxide, did not interfere. After correction for 0.8 per cent free water, this peroxide gave results of 0.0 ± 0.0 per cent. Therefore, the new Karl Fischer procedure when used in conjunction with the specific lead tetraacetate method for hydroperoxides[3] might offer a means of identification and quantitative determination of dialkyl peroxides.

6. Interfering Substances

In addition to alcohols, those classes of compounds which interfered with the Fischer hydroxyl procedure (Chapter IX), namely, aldehydes, cyclohexanone, ortho esters, mercaptans, and acetals, would react in the peroxide method. Based on experience with the determination of ethanol in the presence of diethyl peroxide (page 285, Chapter IX), it was likely that alcohols, ortho esters, and some acetals could be determined in the presence of dialkyl peroxides by the general hydroxyl procedure and, since these reactions were essentially quantitative, it should be possible in turn to correct the apparent peroxide values for these materials. The action of ketones might be catalyzed by peroxides. In this case the method would not be applicable in the presence of these substances.

C. Reactions of Diacyl Peroxides

Diacyl peroxides reacted at least approximately to completion on standing for 30 minutes at room temperature with the hydriodic acid of spent Karl Fischer reagent.[2]

In these experiments Fischer reagent was used which had been charged with sulfur dioxide about one month prior to use. After this time, the rate of degradative side reactions involving the iodine of the reagent was at a minimum (see Chapter III). Consequently, iodine formed by peroxide oxidation of the hydriodic acid apparently was not involved in these parasitic side reactions during the short time of contact.

With benzoyl and probably lauroyl peroxides, the former of which was known to be anhydrous, reaction was evidently quantitative. In other cases, as indicated in Chapter V, approximate analyses for free water were possible, based on the assumption that diacyl peroxides in general reacted to completion with spent Fischer reagent.

A 2.76 millimole sample of benzoyl peroxide (which analyzed 99.8 per cent by the hydriodic acid method—see Chapter V) was dissolved in 25 ml. of ethyl ether. Twenty-five milliliters of exactly discharged Fischer reagent were added and the solution was allowed to stand for 30 minutes at room temperature. The iodine found, based on back titration with standard water-in-methanol, was equivalent to 100.1 ± 0.1 per cent benzoyl peroxide.[2]

Lauroyl peroxide apparently could be titrated for free water. To a 1.144 millimole sample (based on analysis by the hydriodic acid method given in Chapter V), which analyzed 0.00 per cent water by direct titration with Fischer reagent, 5.01 millimoles of water were added. The mixture was titrated directly with Fischer reagent (5.07 millimoles of water found) and then allowed to stand for 30 minutes at room temperature before back titration. Iodine found was equivalent to 1.152 millimoles of peroxide.

Other diacyl peroxides studied appeared to react at a more rapid rate than benzoyl peroxide. Whereas free water could be determined in the latter on rapid direct titration, all other peroxides of this type interfered even at temperatures as low as —40°C. This appeared to be a reasonable basis for assuming that acetyl and succinyl peroxides reacted to completion (see page 143, Chapter V).

CHAPTER XV

Miscellaneous Analytical Procedures

In addition to its use in the general analytical procedures discussed in the previous chapters, the Karl Fischer reagent has been employed in several other methods which were either more limited in scope or not thoroughly investigated.

A. Determination of Acetyl Chloride and Acetylsulfanilyl Chloride

The ease of hydrolysis of acetylpyridinium chloride:

$$C_5H_5N{\cdot}CH_3COCl + H_2O \longrightarrow CH_3COOH + C_5H_5N{\cdot}HCl \qquad (1)$$

was made the basis of an acidimetric procedure for the determination of water by Smith and Bryant.[1] By the use of excess water, this same general reaction has been made the basis of methods for the quantitative determination of acetyl chloride[2] and acetyl-sulfanilyl chloride.[3] (The latter, a sulfonamide drug intermediate, has the formula, $CH_3CONH{\cdot}C_6H_4{\cdot}SO_2Cl$.) Because of the great spread between the vapor pressures of these two compounds, the conditions developed for their quantitative hydrolyses were quite different.

1. DEVELOPMENT OF METHOD FOR ACETYL CHLORIDE

Solutions of approximately 1.5 *M* acetyl chloride were prepared by dissolving about 118 ml. of the technical grade material in toluene or dioxane, respectively, and diluting to 1 liter. Fifteen milliliter portions were transferred to 250 ml. glass-stoppered volumetric flasks. The flasks were cooled in ice and 3 ml. of C.P. pyridine were added carefully.

---

[1] Smith, D. M., and Bryant, W. M. D., *J. Am. Chem. Soc.*, 57, 841–5 (1935).

[2] Smith, D. M., Bryant, W. M. D., and Mitchell, J., Jr., paper presented before the Division of Physical and Inorganic Chemistry, 100th Meeting of the American Chemical Society, Detroit, Mich., September, 1940.

[3] Seaman, W., Norton, A. R., Woods, J. T., and Massad, E. A., *Ind. Eng. Chem., Anal. Ed.*, 16, 517–9 (1944).

Because of its high vapor pressure at room temperature, it was necessary to stabilize the acetyl chloride to prevent significant losses. This was accomplished conveniently by forming the stable, but insoluble, pyridinium salt.

A weighed quantity of water was added. The flasks were stoppered, placed on a mechanical shaker, and agitated for 10 minutes at room temperature. The excess water was determined by titration with Fischer reagent.[4] Total acidity was determined acidimetrically on separate samples prepared at the same time. Results are given in Table CXXXV. The acidimetric results included any free acid present in the acetyl chloride. It was not surprising that these data were slightly higher than the hydrolysis values since some acid would be expected in the technical grade acetyl chloride. The precision as indicated by the duplicate results of experiments 3 and 4 was about ±0.1 per cent; reproducibility on multiple determinations probably would be closer to ±0.2 per cent.

TABLE CXXXV

Determination of Acetyl Chloride by Hydrolysis[4]

No.	Solution	Millimoles of water			Millimoles of acetyl chloride, calculated from acidimetric titration
		Added[a]	Found	Net	
1	Toluene	32.11	6.83	25.28	25.84
2	Dioxane	30.71	7.11	23.60	23.96
3	Toluene	30.10	6.75	23.35	23.73
		30.90	7.57	23.33	23.71
4	Dioxane	32.00	8.27	23.73	23.87
		29.62	5.94	23.68	23.83

[a] Including water in solvent and pyridine.

The effect of time of agitation was studied on more concentrated solutions. (In this organic system involving the insoluble pyridinium salt, hydrolysis became a heterogeneous reaction which was facilitated considerably by continuous agitation.) Fifty milliliter portions of approximately 2 *M* acetyl chloride in dioxane, treated with 15 ml. of pyridine and water as above, were apparently hydrolyzed completely after 15 minutes shaking at room temperature:[4]

---

[4] Unpublished results from the authors' laboratory.

Shaking time, minutes	Water, millimoles		
	Added	Found	Net
15	129.66	17.87	111.79
20	128.58	16.77	111.81
30	131.07	18.89	112.18

2. PROPOSED ANALYTICAL PROCEDURE FOR ACETYL CHLORIDE

This proposed procedure is based on the experiments carried out in the development of the method. The data obtained were sufficiently complete to indicate, without doubt, that this analytical procedure was reliable. No analyses have been made other than those recorded in the above tables.

The sample, containing up to 25 millimoles of acetyl chloride, is weighed into a 250 ml. glass-stoppered volumetric flask containing 20 ml. of C.P. toluene or purified dioxane. The flask is cooled in ice and 15 ml. of C.P. pyridine are added carefully. The flask is shaken and removed from the ice bath. Then 0.55 to 0.6 gram (30.5 to 33 millimoles) of water is weighed into the mixture. (If more convenient the water can be added in pyridine or dioxane solution, in which case a known volume is used and a blank is employed.) The flask is stoppered firmly and agitated on a mechanical shaker for 10 to 15 minutes at room temperature. At the end of this time the mixture is titrated with Fischer reagent.

The total millimoles of water added less those found are equivalent to the millimoles of acetyl chloride in the sample. Total water added is the sum of the moisture contents of the liquids (20 ml. solvent plus 15 ml. of pyridine) as determined by titration, plus the water weighed in, if any.

3. ANALYTICAL PROCEDURE FOR ACETYLSULFANILYL CHLORIDE[3]

A 1 to 2 gram sample (equivalent to 4 to 8 millimoles of acetylsulfanilyl chloride) is weighed into a dry 125 ml. Erlenmeyer flask, which is stoppered at once with a two-hole rubber stopper. One hole is fitted with a drying tube while the other is connected to a desiccant-protected buret containing 1.5 per cent water in pyridine reagent. The flask is cooled in an ice bath and 10 ml. of the reagent are added. (By cooling the flask no moisture is lost through local overheating during the exothermic formation of the pyridinium salt.) Then the flask is withdrawn from the buret, the hole in the stopper is plugged, and the container is swirled gently until the sample is dissolved, after which the flask is removed from the ice bath and the solution is allowed to stand for about 10 minutes to return to room temperature. Twenty-five milliliters of dry methanol are added. (The methanol is added to dilute the yellow color of the solution, permitting easier observation of the visual endpoint.) The resulting solution is titrated immediately with Karl Fischer reagent. A control, containing 10 ml. of the aqueous pyridine reagent and 25 ml. of the methanol, is run with the sample.

Free water is determined by dissolving 2 to 2.5 grams of sample in 25 ml. of dry methanol of known water content and titrating immediately with Fischer reagent. In the absence of an acid acceptor, the acid chloride hydrolyzes (and apparently esterifies) quite slowly. However, in the determination of free water there is some hydrolysis which is proportional to the total water present.

The interfering hydrolysis during titration for free water is probably due to the catalytic activity of the pyridine in Karl Fischer reagent. The correction might be minimized or even eliminated if a large excess of acetic acid were used in place of methanol as solvent for the sample, since in this case the solution would remain acidic throughout the titration.

The initial water concentration is found by the following relation:

$$\text{Per cent water} = \frac{T(1.05\,K - L)}{\text{weight of sample}} \times 100$$

where T = water equivalence of Fischer reagent (grams of water per milliliter); K = total milliliters of Fischer reagent for sample + 25 ml. of methanol; and L = milliliters of Fischer reagent for 25 ml. of methanol.

This represents an approximation of the published equation:[3]

$$\text{Per cent water} = \frac{T(1.0473\,K - L) - 0.298 \times 10^{-3}}{\text{weight of sample}} \times 100$$

but the difference in results introduced by the simplified equation appears to be within the probable experimental error.

The acetylsulfanilyl chloride (M.W. = 233.67) content of the sample was equivalent to the water consumed:

$$\text{Per cent } CH_3CONH{\cdot}C_6H_4{\cdot}SO_2Cl = \frac{[(A + B) - C]\,233.67}{\text{weight of sample} \times 1000} \times 100$$

where A = millimoles of water in control (10 ml. of pyridine reagent + 25 ml. of methanol); B = millimoles of water originally present in the sample; and C = millimoles of water found after reaction.

4. Discussion of Procedure for Acetylsulfanilyl Chloride

The correction for water content was determined from a series of experiments in which known quantities of water were added to acetylsulfanilyl chloride twice recrystallized from chloroform. (The water content, uncorrected, of this sample was 0.019% at the start of the series and 0.036% at its conclusion.) Portions of the sulfonyl chloride were weighed into an Erlenmeyer flask. Then 25 ml. of dry methanol were added, and the flask was stoppered and swirled until the sample was dissolved. A known weight of water was added and the mixture was titrated immediately with Karl Fischer reagent. Analytical results are given in Table CXXXVI.

TABLE CXXXVI

Determination of Error in Analysis for Water[3]

Acetylsulfanilyl chloride added, g.	Total water present,[a] mg.	Total water found, mg.	Net water lost, mg. (present − found)
2.594	3.0	3.0	0.0
2.589	3.3	3.0	0.3
2.096	3.3	3.8	−0.5
2.496	6.1	6.3	−0.2
2.717	7.5	7.7	−0.2
2.277	8.3	8.2	0.1
2.721	12.5	12.2	0.3
2.021	12.7	12.4	0.3
2.432	13.3	13.1	0.2
1.961	15.2	14.6	0.6
1.770	15.6	15.2	0.4
2.163	15.9	15.3	0.6
1.927	16.0	15.4	0.6
2.075	18.2	18.0	0.2
2.358	20.8	19.9	0.9
2.464	22.8	22.1	0.7
1.974	25.5	24.7	0.8
1.952	33.3	32.4	0.9
2.263	44.4	42.3	2.1
2.173	60.1	57.0	3.1
2.098	71.1	68.4	2.7

[a] The values include water found in the sample originally and in the methanol plus the water added.

On the basis of a least squares analysis of these data, the authors[3] assumed that the correlation between water lost and total water found could be expressed as a linear function using column 3 of Table CXXXVI as the independent variable.

However, in Table CXLIV within the probable experimental error the average net values for column 4 corresponding to 18 milligrams or less of water in column 3 are equivalent to about 0.1 ml. of Fischer reagent.

No experimental data were given for the over-all analysis of samples of acetylsulfanilyl chloride, although the authors[3] indicated that some samples gave values close to 100 per cent.

5. INTERFERING SUBSTANCES IN PROCEDURE FOR ACETYLSULFANILYL CHLORIDE

No interference was encountered from the free acids by hydrolysis of the chloride. In general, acids containing no other reactive groups would not interfere.

Sulfanilic acid interfered with the analysis, presumably by acylation of the amine group:

$$HO_3S{\cdot}C_6H_4{\cdot}NH_2 + ClO_2S{\cdot}C_6H_4{\cdot}NHCOCH_3 \longrightarrow HO_3S{\cdot}C_6H_4{\cdot}NHO_2S{\cdot}C_6H_4{\cdot}NHCOCH_3 + HCl \qquad (2)$$

Various quantities of acetylsulfanilyl chloride and sulfanilic acid were weighed into dry Erlenmeyer flasks. Then 10 ml. of aqueous pyridine reagent were added, following the conditions of the analysis. The effects of sulfanilic acid on the analysis are given in Table CXXXVII. (Sulfanilic acid was not present in appreciable amounts in several samples of the sulfonyl chloride.[3])

TABLE CXXXVII

Effect of Sulfanilic Acid[3]

Sulfanilic acid added, mM[a]	Acetylsulfanilyl chloride, mM[a] Taken	Found	Consumed	Ratio, chloride consumed to acid added
0.0583	7.006	6.942	0.064	1.10
0.1243	5.853	5.742	0.111	0.89
0.1942	4.243	4.099	0.144	0.74
0.4131	5.079	4.651	0.428	1.04
0.6390	5.923	5.189	0.734	1.15
0.6674	4.380	3.690	0.690	1.03
				Average 0.99

[a] Millimoles.

Acetone in the methanol even in extremely small quantities could not be tolerated. Samples of the acid chloride were dissolved in methanol containing various quantities of acetone and were titrated for free water.[3]

No.	Acetone in methanol, wt. %	Apparent water found, wt. %
1a	0.003	0.06
1b	0.34	0.50
2a	Trace	0.13
2b	0.35	0.79

Apparently sharp endpoints were obtained. The authors[3] indicated that the high values found in the presence of acetone might be due to ketal formation, catalyzed by the sulfonyl chloride.

The molar increase in water found tends to corroborate this interfering reaction. In experiment No. 2b, for example, assuming 2.5 grams of sample in 25 ml. of solvent, an increase of about 1 millimole of water was found in the presence of 1.2 millimoles of acetone.

6. GENERAL PROCEDURE FOR ACYL HALIDES

Conditions could be devised for the quantitative hydrolysis of most acyl halides. For the higher boiling materials a salt-formation step (for example, combination with pyridine) probably would not be necessary, since volatility would no longer be an important factor, but the presence of pyridine might still be necessary as a catalyst. In some cases the conditions employed for the determination of anhydrides (Chapter XI), using the sodium iodide–aqueous pyridine reagent, might be desirable. In general, however, less drastic conditions should effect complete hydrolysis, since the acyl halides usually are more active than the corresponding acid anhydrides.

B. Determination of Esters

1. DEVELOPMENT OF METHOD

After a study of several caustic media, conditions were found for the complete saponification of a wide variety of esters.[4] Because of the limited quantity of water and alkali which could be tolerated, however, the required conditions were more drastic than those for some acidimetric procedures.[5,6] The reaction was:

[5] Bryant, W. M. D., and Smith, D. M., *J. Am. Chem. Soc.*, **58**, 1014–7 (1936).

[6] Mitchell, J., Jr., Smith, D. M., and Money, F. S., *Ind. Eng. Chem., Anal. Ed.*, **16**, 410–12 (1944).

TABLE CXXXVIII

Analytical Data for Saponification of Esters[4]

Ester	Per cent saponified								
	1 *N* sodium hydroxide in 99% methanol			1 *N* sodium hydroxide in 99% butanol			1 *N* potassium hydroxide in diethylene glycol		
	0.5 hr., 60°C.	1 hr., 60°C.	2 hr., 60°C.	0.5 hr., 100°C.	1 hr., 100°C.	2 hrs., 100°C.	0.5 hr., 107°C.	0.5 hr., 112°C.	0.5 hr., 128°C.
Methyl acetate	96	100	100						
Ethyl propionate	88	98	100				100	100	100
Isobutyl acetate	97	100	100						
Isobutyl isobutyrate		66	73	94	97	100	94	98	100
Ethyl citrate									100
Cyclohexyl acetate	97	100	100						100
Phenyl acetate	80	85	90	100	100	100	98	100	100

$$\mathrm{RCOOR'} + \mathrm{MOH} \longrightarrow \mathrm{RCOOM} + \mathrm{R'OH} \qquad (3)$$

based on the fact that the alkali metal hydroxides titrated mole for mole with the Karl Fischer reagent (Chapter VIII).

Solutions were prepared of 1 *N* sodium hydroxide in 99 per cent methanol–1 per cent water and in 99 per cent *n*-butanol–1 per cent water and of 1 *N* potassium hydroxide in diethylene glycol.

The last reagent was recommended by Redemann and Lucas[7] in their general acidimetric saponification procedure. Potassium hydroxide was used because of its greater solubility over sodium hydroxide in diethylene glycol.

Various esters were saponified with one or more of these reagents under the conditions noted in Table CXXXVIII, finally titrating the excess alkali (and water) with Karl Fischer reagent.

On the basis of these data alicyclic and straight chain aliphatic acetates tended to react quantitatively with the caustic in methanol within 1 hour at 60°C. The less reactive esters studied were saponified after 2 hours at 100°C. with caustic in butanol or 0.5 hour at 128°C. with the diethylene glycol reagent. The last named medium appeared to be the most desirable from the standpoints of speed and general applicability.

2. Reagents

One normal potassium hydroxide in technical grade diethylene glycol is prepared by dissolving 14.5 grams of potassium hydroxide pellets, weighed into a 250 ml. glass-stoppered volumetric flask, in about 100 ml. of the glycol at 60°C. After cooling to room temperature, the solution is diluted to 250 ml. with more of the glycol. (When prepared in this way the final reagent is only lightly colored; if the potassium hydroxide is dissolved in the total volume of the glycol a much darker solution is obtained.)

3. Analytical Procedure

The sample, containing up to 5 milliequivalents of ester plus acid, is weighed into a 250 ml. glass-stoppered volumetric flask with a grooved stopper. (A groove cut around the stopper when filled with a high melting point stopcock grease provides an effective seal at elevated temperatures; see page 399, Chapted XVI.) Ten milliliters of reagent are added, and the lubricated stopper fastened with a

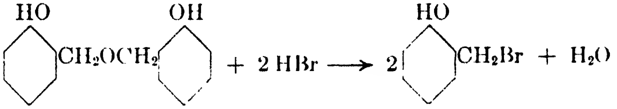
---

[7] Redemann, C. E., and Lucas, H. J., *Ibid.*, 9, 521–2 (1937).

spring clamp. A flask containing 10 ml. of reagent is also prepared as a control. The vessels are placed in an oven at 128 ± 2°C. for 30 minutes, at the end of which time they are removed, allowed to cool to room temperature, and the contents are titrated with Fischer reagent. The net millimoles of apparent water found (blank — sample) plus the millimoles of water originally present in the sample are equivalent to the ester content of the sample. (Methods for the determination of free acid content are described in Chapter X.)

C. Determination of Dibenzyl Ether Linkages in Phenol Alcohols and Derived Phenolic Resins

The structures of ethers of this type usually were established by cleavage with gaseous hydrogen bromide, followed by isolation and analysis of the bromomethyl compound produced, for example:



(4)

Methods for the determination of this brominated product, based on bromine analysis by some combustion method, were reported to be subject to many difficulties. The hydrogen bromide had to be free of bromine and also had to be eliminated completely from the product before analysis. Often the bromomethyl compound was subject to spontaneous decomposition.[7a]

Lilley and Osmond [7a] found that most of these problems were eliminated by determining the water formed in reaction (4) by titration with Fischer reagent. They employed chloroform as solvent. In all methods based on reaction (4), any methylol groups present in the sample would be converted to the bromide:

$$RCH_2OH + HBr \longrightarrow RCH_2Br + H_2O \tag{5}$$

and would require a stoichiometric correction to the total water (or bromine) found. A suitable independent iodometric procedure employing aqueous iodine was recommended[7b]:

[7a] Lilley, H. S., and Osmond, D. W. J., *J. Soc. Chem. Ind.*, **66**, 425–7 (1947).

[7b] Lilley, H. S., and Osmond, D. W. J., *Ibid.*, **66**, 340–1 (1947).

$$RCH_2OH + 2\,I_2 + H_2O \longrightarrow RI + HCOOH + 3\,HI \qquad (5a)$$

The authors[7a] noted that the fission of ethers by hydrogen bromide appeared to be rapid but that bromination of methylol groups was relatively slow, requiring as much as 24 hours at room temperature.

1. Reagents

Dry chloroform is obtained by drying over calcium for 3–4 days. The Karl Fischer reagent used is equivalent to about 2 milligrams of water per milliliter.

2. Apparatus

The apparatus requirements, listed in the experimental order, include: (*a*) a generator for gaseous hydrogen bromide from potassium bromide and orthophosphoric acid; (*b*) a large Buchner flask immersed in cold water to trap water from (*a*); (*c*) a red phosphorus trap for bromine, if any; (*d*) two absorption towers of 14-mesh calcium chloride; (*e*) two towers of anhydrone ("Drierite" probably would be satisfactory); (*f*) the reaction vessel, a 75 ml. flask containing 40 ml. of dry chloroform; (*g*) two additional 75 ml. flasks, each with 40 ml. of dry chloroform, to entrap any water of reaction carried over during the analysis; (*h*) methanol–solid carbon dioxide baths for vessels (*f*) and (*g*); (*i*) vacuum pump.

3. Analytical Procedure

A blank on the apparatus is run first to insure that no water is brought over from the HBr generator, followed by a control on a known weight of water added to the reaction flask (*f*), containing 40 ml. of chloroform. Then about 300 milligrams (accurately weighed) of sample is placed in flask (*f*), containing 40 ml. of fresh chloroform. A slow stream of hydrogen bromide is drawn through the apparatus by means of a vacuum pump. After 30 minutes, the hydrogen bromide addition is terminated. The trapping vessels (*g*) are titrated for water immediately but the reaction vessel (at least with unknown samples) is allowed to stand for 12–24 hours at room temperature before titration to insure complete reaction of any methylol groups likely to be present.

After correction for methylol content[7b] and for free water originally present in the sample, the net Fischer reagent titer is equivalent mole for mole to the ether link content of the sample.

4. Analytical Results

The method was checked on several carefully prepared dibenzyl ethers. The results are shown in Table CXXXIX.

TABLE CXXXIX

Determination of Dibenzyl Ethers[7a]

Ether	Ether link as $—CH_2OCH_2—$, wt. %	
	Calculated	Found
Bis(2,6-dibromo-3,5-dimethyl-4-hydroxybenzyl)	7.67	7.5 ± 0.1
Bis(3,5-dimethyl-4-hydroxybenzyl)	15.38	15.2 ± 0.1
Bis(3,5-dichlorohydroxybenzyl)	12.02	12.25 ± 0.05
Bis(3-bromo-5-methyl-2-hydroxybenzyl)	11.35	11.2 ± 0.0
Bis(3-cyclohexyl-2-hydroxy-5-methylbenzyl)	11.17	11.0 ± 0.1
Bis(5-*tert*-butyl-2-hydroxy-3-methylbenzyl)	13.84	13.7 ± 0.1
Bis[2-hydroxy-3-(2-hydroxy-5-methylbenzyl)-5-methylbenzyl]	8.83	8.8 ± 0.05

Several phenolic resins then were analyzed by the Fischer reagent method and the values obtained were compared with total bromine results and with ultimate analyses (Table CXL).

TABLE CXL

Determination of Ether Linkages in Phenolic Resins[7a]

Resin	Ether link as $—CH_2OCH_2—$, wt. %		
	Fischer reagent method	Bromine method	Ultimate analysis[a]
1[b]	2.2 ± 0.2	2.4	2.2
2[b]	0.55 ± 0.05	0.55	0.6
3[b]	2.8 ± 0.0	2.7	2.6
4[c]	9.7 ± 0.3		9.8
5[c]	1.0 ± 0.2		1.1

[a] Calculated from combustion data; all oxygen present as ether linkages.
[b] Ammonia catalyzed resins.
[c] Soda catalyzed resins.

Lilley and Osmond[7a] indicated that the results by their Fischer reagent procedure helped to clarify the structure of phenolic resins, their behavior (with respect to structure) toward the action of heat and the mechanism of their combination with unsaturated substances.

D. Determination of Mercaptans

In Chapter V the authors indicated that mercaptans were oxidized readily to the disulfides by the iodine of Karl Fischer reagent:

$$2\,RSH + I_2 \longrightarrow RSSR + 2\,HI \qquad (6)$$

In many cases this reaction was quantitative. When used in conjunction with the new boron trifluoride catalyzed olefin addition reaction for the removal of mercaptan interference in the determination of water (see page 135 ff., Chapter V), the Fischer reagent titration permitted a calculation of the mercaptan content of the sample. Several mercaptans in 25 ml. of methanol solution were analyzed for water and mercaptan in the presence and absence of added water. Comparative analyses for the thiol group were made either by the iodimetric[8] or silver nitrate method.[9] Analytical data are given in Table CXLI. With the exception of hexylmercaptan, which was fractionated, the compounds were analyzed as received. (Thiobenzanilide, an interfering substance, reacted partially; values of 16.8 ± 0.2 per cent were found by direct titration.)

E. Determination of Metal Salts of Organic Acids

1. General

Alkali metal salts of organic acids usually could be titrated potentiometrically in organic or aqueous media with standard acid, the titer serving as a measure of total metal ion. Metal salts of organic acids (with the exception of aromatic acids) were found to react quantitatively with methanol containing 100 grams of boron trifluoride per liter (general carboxyl catalyst; Chapter X).[4] The course of the reaction could be represented in two different ways:

$$RCOOM + CH_3OH \xrightarrow{BF_3} RCOOCH_3 + (BF_3)_xMOH \qquad (7a)$$

---

[8] Kimball, J. W., Kramer, R. L., and Reid, E. E., *J. Am. Chem. Soc.*, 43, 1199–1200 (1921).

[9] Borgstrom, P., and Reid, E. E., *Ind. Eng. Chem., Anal. Ed.*, 1, 186–7 (1929).

TABLE CXLI

Analytical Data for Mercaptans and Other Thiol Compounds[4]

Substance		Mercaptan found, wt. % (other method)	Water added, millimoles	Complete analysis using Fischer reagent, wt. %		
Name	Millimoles			Mercaptan	Water	Total
Ethyl mercaptan	5.6	99.1[a]	0.0	98.8	0.5	99.3
Isopropyl mercaptan	6.2	94.3[a]	0.0	94.1	0.9	95.0
	6.5		8.2	94.1[c]		
Butyl mercaptan	10.0	92.6,[a] 92.5[b]	0.0	92.2	0.8	93.0
	8.5		0.9	92.7[c]		
	8.5		8.2	92.5[c]		
Isoamyl mercaptan	6.0	98.3[b]	0.0	98.7	0.4	99.1
Hexyl mercaptan	7.2	99.2,[a] 99.8[b]	0.0	99.7	0.2	99.9
Heptyl mercaptan	6.8	97.9[b]	0.0	98.7	0.7	99.4
Thiophenol	7.2	95.0[a]	0.0	95.8	0.3	96.1
Benzyl mercaptan	6.6	98.7[a]	0.0	98.4	0.3	98.7
	6.6		8.2	98.0[c]		
Thioglycolic acid	9.5	93.1[a]	0.0	94.0	3.8	97.8
Ethyl thioglycolate	10.2	98.9[a]	0.0	99.0	0.5	99.5
	10.2		6.8	98.8[c]		
Thio-β-naphthol	7.4	99.8	0.0	100.0	0.1	100.1
	7.4		6.8	100.2[c]		

[a] By iodimetric method.[8]
[b] By silver nitrate titration.[9]
[c] Net Fischer reagent titer after correcting for original water found plus water added.

$$MOH + I_2 + SO_2 + CH_3OH \longrightarrow MI + HI + HSO_4CH_3 \quad (7b)$$

or:

$$RCOOM + CH_3OH \xrightarrow{BF_3} RCOOH + (BF_3)_x{\cdot}MOCH_3 \quad (8a)$$

$$RCOOH + CH_3OH \longrightarrow RCOOCH_3 + H_2O \quad (8b)$$

The net reaction with respect to Fischer reagent would be the same in either case.

It was interesting that salts of aromatic acids, in common with the free acids, reacted only partially.

Boron trifluoride alcoholates are reported to be highly ionized and to behave as strong acids:[10-12]

$$BF_3{\cdot}ROH \rightleftharpoons BF_3{\cdot}RO^- + H^+ \quad (9)$$

Boron trifluoride–alkoxide complexes, e.g., $BF_3{\cdot}CH_3ONa$, are reported to form readily.[13] The authors observed that a solution of boron trifluoride in methanol effectively eliminated interference of sodium methylate in the Fischer reagent titration. Quantitative titration of metal hydroxides in the presence of boron trifluoride also was practical with Fischer reagent.[4]

2. Reagent

Methanol containing about 100 grams of boron trifluoride per liter is prepared by diluting 195 grams (150 ml.) of $BF_3{\cdot}2CH_3OH$ to 1 liter with methanol. (See page 300, Chapter X, for preparation of $BF_3{\cdot}2CH_3OH$.) C.P. pyridine is also used.

3. Analytical Procedure

The sample, containing up to 10 milliequivalents of salt, is weighed directly into a 250 ml. glass-stoppered volumetric flask. Then 20 ml. of methanol reagent are added. The flask, together with a blank, is placed in a water bath at 60 ± 1°C. After being momentarily loosened to allow for expansion of the enclosed air, the stoppers are tightened. The flasks are left in the bath for 1 hour, and are then removed and allowed to cool to room temperature spontaneously. Five milliliters of pyridine are added carefully and the solutions are titrated directly with Karl Fischer reagent.

Free water is determined by direct titration of the sample dispersed in 25 ml. of dry methanol (see Chapter VII). The net increase in titer (sample—blank) less the water originally present in the

---

[10] Bowlus, H., and Nieuwland, J. A., *J. Am. Chem. Soc.*, **53**, 3838–40 (1931)

[11] O'Leary, L. A., and Wenzke, H. H., *Ibid.*, **55**, 2117–21 (1933).

[12] Meerwein, H., *Ber.*, **B66**, 411–14 (1933).

[13] Meerwein, H., and Pannwitz, W., *J. prakt. Chem.*, **141**, 123–48 (1934).

sample is a quantitative measure of the metal salt plus free organic acid content of the sample.

4. Experimental Results

Analytical data for several c.p. salts, most of which were hydrated, are given in Table CXLII. None of these compounds were subject to oxidation-reduction reactions when titrated with Fischer reagent. (For compounds subject to these types of reactions suitable corrections probably could be applied; see Chapter VIII.)

TABLE CXLII

Analytical Data for Metal Salts of Organic Acids[4]

No.	Salt	Complete analysis using Karl Fischer reagent, wt. %		
		Salt[a]	Water	Total
1	Sodium formate	(2) 99.9 ± 0.2	0.1 ± 0.0	100.0
2	Ammonium acetate	(2) 95.7 ± 0.6	0.9 ± 0.2	96.6
3	Barium acetate	(4) 93.9 ± 0.9	2.1 ± 0.0	96.0
4	Cadmium acetate	(2) 85.1 ± 0.3	13.4 ± 0.0	98.5
5	Cobalt acetate	(2) 70.6 ± 0.2	28.9 ± 0.1	99.5
6	Cuprous acetate	(2) 87.5 ± 0.0	5.2 ± 0.2	9234
7	Lead acetate	(2) 85.5 ± 0.2	13.8 ± 0.0	99.7
8	Magnesium acetate	(2) 66.5 ± 0.6	33.9 ± 0.2	100.8
9	Mercuric acetate	(2) 92.4 ± 0.5	3.7 ± 0.2	96.1
10	Nickel acetate	(2) 70.9 ± 0.4	28.7 ± 0.1	99.6
11	Silver acetate	(2) 78.6 ± 0.1	2.2 ± 0.0	80.8
12	Sodium acetate	(8) 60.4 ± 0.2	39.4 ± 0.2	99.8
13	Strontium acetate	(2) 95.2 ± 0.2	3.8 ± 0.0	99.0
14	Uranyl acetate	(4) 92.6 ± 0.1	8.4 ± 0.0	101.0
15	Zinc acetate	(3) 81.9 ± 0.4	16.4 ± 0.0	98.3
16	Ammonium oxalate	(2) 87.6 ± 0.2	11.2 ± 0.1	98.8
17	Calcium propionate	(4) 88.9 ± 0.3	8.3 ± 0.0	99.3
18	Calcium malonate	(2) 81.5 ± 0.0	18.3 ± 0.2	99.7
19	Sodium succinate	(6) 62.8 ± 0.6	36.5 ± 0.5	99.3
20	Ammonium citrate	(2) 94.6 ± 0.2	0.2 ± 0.0	94.8
21	Sodium citrate	(2) 86.6 ± 0.3	11.7 ± 0.2	98.3
22	Calcium lactate	(2) 72.2 ± 0.6	28.5 ± 0.2	100.7
23	Sodium tartrate	(2) 70.6 ± 0.1	15.3	85.9
24	Potassium bitartrate	(2) 84.9 ± 0.4	0.2 ± 0.0	85.1
25	Zinc stearate	(2) 99.8 ± 0.3	0.2 ± 0.0	100.0
26	Sodium cyclohexane carboxylate	(2) 95.6 ± 0.3	0.1 ± 0.0	95.7

[a] Figures in parentheses represent number of individual determinations.

Basic aluminum acetate and potassium oxalate appeared to be completely insoluble in the reagent. Analyses of 17 and 30 per cent, respectively, were obtained. (The "Hydractor"—page 198, Chapter VI—might aid in the analysis of insoluble compounds.) The salts of aromatic acids, sodium *m*-nitrobenzoate and potassium acid phthalate, although soluble, gave values of 65 and 60 per cent, respectively.[4]

F. Determination of Sulfur Trioxide in Fuming Sulfuric Acid

1. GENERAL

Sulfur trioxide in sulfuric acid usually was determined indirectly by alkali titration. Parker[14] based his method on direct titration with water until the sample no longer fumed in air. Brand[15] modified this method by reducing the temperature of the titration from below 45° to below 13°C. to minimize volatility losses.

Axford and Sugden[16] determined sulfur trioxide (at about 2 per cent concentration) in sulfur dioxide by absorption of the sample in sodium hydroxide, followed by acidification and evolution of the sulfur dioxide in a stream of nitrogen. After neutralization, the residue was titrated amperometrically with lead nitrate solution.

A Karl Fischer procedure was developed, based on hydrolysis:

$$H_2SO_4{\cdot}SO_3 + H_2O \longrightarrow 2\ H_2SO_4 \qquad (10)$$

followed by titration of the excess water with Fischer reagent.[4]

2. REAGENTS

Concentrated sulfuric acid containing about 4 per cent water, and a solution of equal volumes of C.P. pyridine and technical dioxane are used.

3. ANALYTICAL PROCEDURE

Exactly reproducible amounts (approximately 2 ml.) of concentrated sulfuric acid reagent are delivered from a precision pipet (Fig. 49) into a 250 ml. glass-stoppered volumetric flask. Then 2.0 to 2.5 grams of sample are weighed into the flask, taking care that the sample drops directly into the acid.

The ratio of sample taken to water added is based on a sulfur trioxide content of no more than 25 per cent. Higher concentrations would require correspondingly larger portions of acid reagent or, preferably, smaller samples. The specified quantities allow only about a 1 millimole excess of water (exclusive

---

[14] Parker, R. G., *J. Soc. Chem. Ind.*, **36**, 692–4 (1917).
[15] Brand, J. C. D., *J. Chem. Soc.*, **1946**, 585–8.
[16] Axford, D. W. E., and Sugden, T M., *Ibid.*, **1946**, 901–3.

of moisture in the pyridine–dioxane reagent) for samples containing 25 per cent sulfur trioxide.

The flask is cooled in ice and 30 ml. of cold pyridine–dioxane reagent are added.

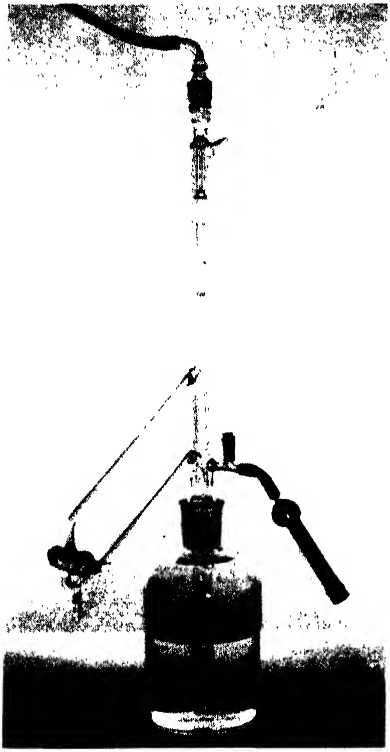


Fig. 49. Precision pipet.[4]

Pyridine is added to combine with the sulfuric acid to eliminate a false endpoint during the subsequent Fischer reagent titration and dioxane is added to act as a solvent. In the absence of dioxane, the pyridine salt which forms is quite hard and difficult to disperse and dissolve during titration.

The mixture is allowed to warm to room temperature spontaneously and is then titrated directly with Karl Fischer reagent. Total water added is determined by titrating a blank containing 2 ml. of sulfuric acid reagent plus 30 ml. of the pyridine–dioxane solution.

The millimoles of water consumed (blank minus sample titer) are equivalent to the sulfur trioxide content of the sample.

4. EXPERIMENTAL RESULTS

A sample of fuming sulfuric acid, analyzing 21.5 per cent sulfur trioxide by acidimetric titration, gave values of 22.3 ± 0.2 per cent for quadruplicate determinations.

This new Karl Fischer reagent procedure should be equally effective in the determination of sulfur trioxide in other materials, e.g., sulfur trioxide in sulfur dioxide.

5. OTHER STUDIES

Attempts to hydrolyze the sulfur trioxide directly in the cold (ice bath) with an aqueous pyridine–dioxane solution were unsuccessful. Some sample always was lost during the exothermic hydrolysis-neutralization step. The maximum value on the fuming sulfuric acid which analyzed 21.5 per cent sulfur trioxide by acidimetric titration was only 14.5 per cent.

Low results were also obtained on using sodium acetate trihydrate to supply the water for hydrolysis.

CHAPTER XVI

Proposed Further Studies Involving Karl Fischer Reagent

A. Possible Modified Reagents (Chapters II and III)

The Karl Fischer reagent, composed of iodine, sulfur dioxide, and pyridine in methanol, has the outstanding advantage of permitting titration to a visual endpoint in the majority of cases, with the reagent acting as its own indicator. The principal deficiency lies in its instability. Because of degradative side reactions, the reagent tends gradually to lose its effective strength, requiring at least daily standardization. (Some batches of reagent appear to be more stable than others.) As indicated in Chapter III, combinations of iodine and sulfur dioxide with other amines in place of pyridine, and other solvents in place of methanol, have been thoroughly investigated. However, to the authors' knowledge, no work has been reported on the use of a fifth component to inhibit these side reactions. Also, no investigations have been published on modified reagents employing other active ingredients. For example, it would be interesting to study bromine reduction, which in aqueous solution reacts in a manner analogous to that of iodine:

$$Br_2 + SO_2 + 2\,H_2O \longrightarrow 2\,HBr + H_2SO_4 \qquad (1a)$$

Bromine and nitrous acid could react with water:

$$Br_2 + HNO_2 + H_2O \longrightarrow 2\,HBr + HNO_3 \qquad (1b)$$

In this system pyridine might not be satisfactory as an acid acceptor because of possible bromine addition. The use of a saturated tertiary amine, such as tributylamine, might be feasible.

An interesting modified reagent was developed by Johansson,[1a] which should be of value for those laboratories where water determinations are made only occasionally. He used two solutions similar

---

[1a] Johansson, A., *Svensk Papperstidn.*, **50**, No. 11B, 124–6 (1947).

to those offered by some of the chemical supply houses. Solution I was prepared in the proportions: 300 ml. of dry methanol, 300 ml. of dry pyridine, and 60 grams of sulfur dioxide. Solution II was methanol containing about 30 g. of iodine per liter. In preparing for the titration of cellulose and related materials, Johansson[1a] added the sample, directly or dissolved in alcohol, to solution I pretitrated with solution II. After a short extraction period (about 20 minutes at room temperature for defibrated pulp and 60 to 90 minutes for undefibrated pulp, cut in 1 × 2 × 20 mm. strips), the resulting mixture was titrated to either the visual or indirect dead-stop endpoint with solution II, which was delivered from a buret. The reagent was standardized in a similar manner; a known quantity of water (about 50 mg.) was added in place of sample.

Results reported on the determination of water in refined cellulose by this technique compared favorably with those obtained by oven drying. For example, unbleached sulfite pulp analyzed 9.29 weight per cent moisture by titration, compared to 9.31 per cent weight loss after 48 hours at 105°C. Bleached sulfite pulp, sulfate pulp, rayon cellulose, sawdust, and defibrated pulp analyzed 6.40, 7.80, 9.95, 8.70, and 6.61 weight per cent, respectively, by titration, compared to 6.47, 7.83, 10.32, 8.67, and 6.49 per cent weight loss after 48 hours at 105°C.

All of the compounds Johansson[1a] studied contained appreciable quantities of water (4.0 to 42.6 per cent). He suggested that his method eliminated the necessity for elaborate protection of the titration equipment from moisture contamination, since neither of his reagents was particularly hygroscopic. It should be noted, however, that an error would be expected from parasitic side reactions which might be noticeable only with samples containing small quantities of water. Studies on the nature of the Fischer reagent (Chapter III) indicated that freshly prepared active reagent was subject to an initially high, unreproducible rate of degradation (see page 54). This error should be more serious when the back-titration (indirect) technique is employed, since free iodine will be present in the mixture for a short time.

B. Direct Electrometric Micro Methods (Chapter IV)

In view of improved direct electrometric macrotitration methods more satisfactory micro techniques should be feasible.

C. Interferences of Organic Compounds (Chapter V)

The study of the effects of organic functional groups on Karl Fischer reagent should be extended to include dithio acids and per acids. In Chapter V the authors suggested that the former would probably be oxidized to the disulfides by the iodine of Fischer reagent and that the latter would rapidly oxidize the hydrogen iodide always present in the solution. If these classes of substances should prove reactive it should be possible to devise means for reacting them to form compounds which are inert toward Fischer reagent. A technique suitable for peracids possibly would be applicable to the more active diacyl peroxides, in which, at present, water can be determined only indirectly. Any reagents employed for these purposes must also be inert toward Fischer reagent in both the oxidized and reduced states. The interference of *l*-ascorbic acid and quinone probably could be eliminated by the use of suitable weak oxidizing or reducing agents which would be inert to Fischer reagent.

D. Applications to Commercial Materials (Chapter VI)

In the commercial field many classes of materials require study. The activities of soluble materials can be predicted with fair certainty provided that the compositions are known; Chapters V, VI, VII, and VIII contained detailed information on the behavior of a wide variety of pure compounds. Substances which require extraction, however, need further evaluation. In some cases suitable solvents may be found; for example, polyglycols might dissolve sugars and malt syrup. When thermal stability is an unknown factor, the applications of low-temperature extractions should be more thoroughly investigated. With sufficiently fine states of division and relatively large volumes of extractant, the use of temperatures at or below that of the room should be feasible in general. Occasionally a second extraction may be necessary. With wood, for example, equilibrium conditions appeared to be reached after 96 to 97 per cent of the moisture had been removed. A second extraction was necessary before essentially all of the water was available for titration. Further investigations on fats, waxes, meat, and leather products would be desirable. Some work has been done in Russia in connection with the use of a modified Fischer reagent (a higher concentration of pyridine apparently was used) for the determination of moisture in leather products,[1b] but up to the present time no details have become available.

Many investigators, as indicated in Chapter VI, attempted extractions using as little as 10 ml. of methanol. Often, 50 to 100 ml. of this or other water-miscible liquid would be more effective. Provided that dry solvents were used (commercially available methanol contains no more than 0.03 per cent water), the blank corrections for these volumes would remain quite small.

E. Quantitative Aspects of Inorganic Reactions (Chapter VIII)

Analytical possibilities in the inorganic field have received only minor attention. The authors have demonstrated the quantitative nature of the reactions of a variety of inorganic substances. Under proper conditions many metal ions could be precipitated and separated as the dry hydroxides (by extraction), oxides, or carbonates, which would titrate stoichiometrically with Karl Fischer reagent. The possibility of precipitating the hydroxides of lead, copper, and barium by the reaction of sodium hydroxide on the acetates of these metals in nonaqueous solution was scouted by the authors:

$$\mathrm{MOOCCH_3 + NaOH \longrightarrow MOH + NaOOCCH_3} \tag{2}$$

Preliminary data indicated that it should be practical, at least in some cases, to recover and dry the hydroxides quantitatively.

After quantitative dehydration to the oxides the resulting compounds usually would react stoichiometrically with Fischer reagent provided that they were not ignited.

A sample of calcium acetate (11 millimoles) was treated with 10 ml. of 23 per cent aqueous sodium hydroxide. The precipitate was recovered and dried at 140°C. Then 10 ml. of glacial acetic acid were added and the mixture was titrated with Fischer reagent. The iodine consumed was equivalent to 94 per cent of the calcium added.

Tetraethyl lead and other materials which reduce iodine or oxidize hydrogen iodide might be analyzed conveniently by titrations involving Fischer reagent.

Four compounds of cobalt of the general formula $[\mathrm{Co(OH)}_a(\mathrm{H_2O})_b(\mathrm{NH_3})_{6-a-b}]\mathrm{X}_{3-a}$ have been titrated with Fischer reagent under various conditions.[2] When suspended in methanol or

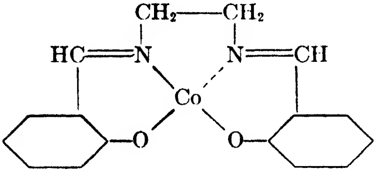
---

[1b] Berkenheim, T. I., *Trudy Moskov. Teknol Inst. Legkoi. Prom. im. L. M. Kaganovicha,* **1941, No. 3, 3–11.**

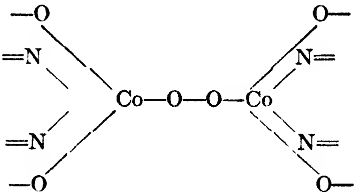
[2] Unpublished results from the authors' laboratory.

boron trifluoride–acetic acid solution, low variable results were obtained, apparently because of incomplete reaction. All four samples were soluble in 96 per cent sulfuric acid; the acid solutions were cooled to 0°C. and excess pyridine was added. (Pyridine hydrogen sulfate precipitated initially but at the endpoint of the Fischer reagent titration the solution was homogeneous.) The mixtures were then titrated with Karl Fischer reagent. The results were closely reproducible but did not correspond to the expected values except in one case; two results were high and one was low. Also the samples were several years old and the chemical analyses did not correspond exactly to the supposed compositions. Although the results were not conclusive, it seemed that the Fischer reagent would be a useful tool in systematic studies of coordination compounds containing water or hydroxyl groups.

The substance, cobaltous bis-salicylalethylenediimine[3] (Salcomine), was studied analytically.* The behavior of this material in its various forms toward the Karl Fischer reagent under different conditions was highly interesting, although it was not worked out completely. Enough of our work is reported here to indicate the possibilities for research with the Fischer reagent.



(I)



(II)

Salcomine (I) had an active form which on exposure to air could absorb up to 1 molecule of oxygen per 2 atoms of cobalt to form a peroxide (II).

When heated, up to the 2 atoms of oxygen were lost from the peroxide, leaving the original cobaltous compound in substantially quantitative yield. On suspension in methanol, however, apparently 1 atom of oxygen was released from the peroxide, leaving an oxide

* This work was done under N.D.R.C. Contract O.S.R.D. 1951.

[3] Calvin, M., Bailes, R. H., and Wilmarth, W. K., *J. Am. Chem. Soc.*, **68**, 2254–6 (1946).

which, on titration with Fischer reagent, reacted with very nearly 0.5 mole of iodine per cobalt atom; when the compound was in the deoxidized (cobaltous) state, the same titration ratio was observed.

When the deoxidized compound was heated at 10^{-5} mm. of mercury for 2 hours at 100–200°C., the same reaction ratio with Karl Fischer reagent was also observed. This apparently eliminated the presence of a hydrate.

Presumably the following reactions took place:

$$2\,(-O-Co-O-) + I_2 \longrightarrow 2\,(-O-Co-O-)^+ + 2\,I^- \qquad (3)$$

$$(-O-Co-O-)_2O + I_2 + SO_2 + CH_3OH \longrightarrow 2\,(-O-Co-O-)^+ + 2\,I^- + CH_3OSO_3H \qquad (4)$$

Reaction (3) was the reaction of coordinated cobaltous ion with iodine to form coordinated cobaltic ion. Reaction (4) represented the behavior of coordinated cobaltic oxide with Fischer reagent, analogous to that of the reactions of many metal oxides (see page 248).

The coordinated cobaltic compound, after removal of the oxide oxygen with alcoholic hydrogen chloride and drying, showed essentially no reaction with Karl Fischer reagent.

In making an analysis of the oxidized material, 2 grams of sample were weighed into a glass-stoppered electrometric titration flask. Then 100 ml. of dry methanol and excess Fischer reagent were added. The flask was connected to the titrimeter apparatus (see page 94, Chapter IV) and the mixture was back titrated with standard water-in-methanol to the "dead-stop" endpoint. For the titration of the deoxidized material, the sample in the titration flask was heated for 1.5 hours at 100°C. under an atmosphere of nitrogen and was then cooled to room temperature in a desiccator under nitrogen before the addition of methanol. Otherwise, the procedure was the same as that for the oxidized compound.

During the first deoxidation step all free water also was lost. Consequently, the net titer between the oxidized and deoxidized materials (allowing for the weight change due to loss of volatiles) became a measure of original free water. [When the deoxidized material was allowed to absorb oxygen, there was no significant change in the Karl Fischer titration ratio (referred to cobalt) for a single cycle.] For example, 10 millimole portions of an oxidized and deoxidized sample analyzed 5.5 ± 0.2 and 4.9 ± 0.1 millimoles of apparent water, respectively. The net value of 0.6 millimole was equivalent to the free water.

There also was evidence of an hydroxy compound when the chelate was prepared from ethanol solution. In one case, the Fischer reagent titer was equivalent to 0.94 mole of iodine per gram atom of cobalt, suggesting the reaction:

$$(-O-Co-O-)OH + I_2 + SO_2 + CH_3OH \longrightarrow (-O-Co-O-)^+ + H^+ + 2\,I^- + CH_3OSO_3H \quad (5)$$

This reaction was analogous to that of the alkali hydroxides (page 249).

In further experiments it was shown that uncoordinated cobaltous ion did not react with the Fischer reagent and that the coordinated cobalt did not react with a Fischer reagent from which the sulfur dioxide was omitted. (This was true as far as the type of cobalt coordination under investigation was concerned.) According to these experiments, cobalt could be coordinated with ethylenediamine, particularly in the presence of oxygen, and with salicylethylenediamide. (Salicylaldoxime, in the presence of air, combined with cobalt to give a compound which showed no net reaction with Karl Fischer reagent.)

F. Quantitative Organic Analytical Techniques (Chapters IX through XV)

The approximate status of the organic analytical procedures based on the Karl Fischer reagent is summarized in Figure 50. In this chart the procedures are arranged according to the classes of compounds for which the methods were designed. The approximate status of each method is shown by the amount of shading in the corresponding block of the figure. Thus, procedures for which detailed studies have been made and for which fairly general procedures are available, are shown as fully shaded areas; methods of limited application or incompletely developed methods are shown as partially shaded areas, and a few procedures which might be evolved—based on analogy to or extrapolation from proven Karl Fischer methods—are shown as unshaded areas. Most of the proven Karl Fischer reagent techniques either offer methods of analysis where none was previously available (for example, the procedure for nitriles) or permit determinations of many combinations of functional groups heretofore extremely difficult or impractical by other methods (for example, complex mixtures containing alcoholic hydroxyl and carboxyl).

Some Karl Fischer reagent techniques are particularly useful when applied in conjunction with other procedures. Thus, direct methods for the resolution of amine mixtures are available when the Karl Fischer procedures for primary and for primary plus secondary amines are used in conjunction with acidimetric procedures for tertiary amines and secondary plus tertiary amines (see pages 338 and 346, Chapter XIII).

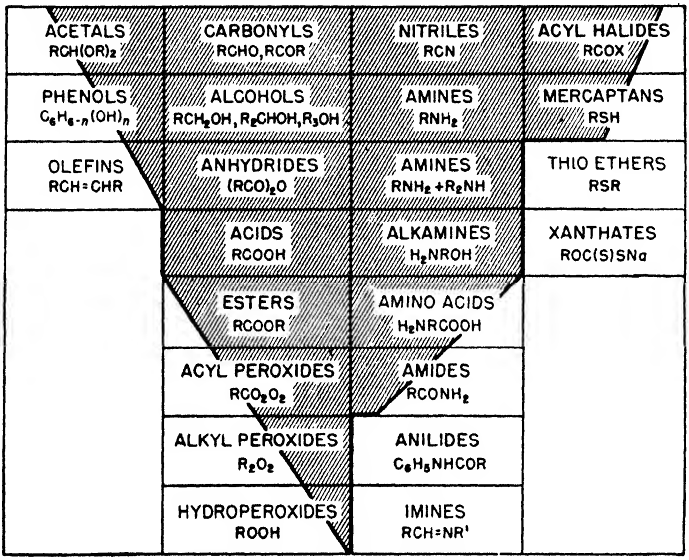


Fig. 50. Status of organic analytical procedures.

The procedure discussed in Chapter V for the addition of mercaptans to olefins, to permit the determination of free water in the former, might be made the basis of a novel titrimetric method of analysis for unsaturates:

$$RCH{=}CHR + R'SH \longrightarrow R(R'S)CHC_2R \qquad (6)$$

In preliminary studies in the authors' laboratory, definite addition was observed when excess hexyl mercaptan was added to octene and cyclohexene, in the presence of boron trifluoride–ethyl ether complex, the extent of reaction being determined by titration for mercaptan before and after reaction.

Mercaptans are oxidized essentially quantitatively to the disulfides on direct titration with Fischer reagent (see Chapter XV).

Hoog and Eichwald[4] removed straight chain aliphatic olefins from hydrocarbon mixtures by reaction with a 300 per cent excess of thioglycolic acid at room temperature, using propionic acid as a homogenizing agent.

General methods for amides and anilides might be evolved which are based on hydrolysis or alcoholysis. However, attempts to hydrolyze acetanilide with an 100 per cent excess of water in the presence of acetic acid containing 100 grams of boron trifluoride per liter were unsuccessful:

$$C_6H_5NH{\cdot}COCH_3 + H_2O \xrightarrow{H^+} C_6H_5NH_2 + CH_3COOH \qquad (7)$$

No water was consumed after heating 10 millimoles of acetanilide with 20 ml. of this catalyst solution containing 20 millimoles of water for 2 hours at 100°C.[2] From 2 to 3 per cent reaction was observed after heating 8 millimoles of acetanilide at 60°C. for 1 to 3 hours with 2.5 ml. of $BF_3{\cdot}CH_3OH$ in 10 ml. of dioxane and then estimating unreacted alcohol by the general hydroxyl procedure (Chapter IX).

$$C_6H_5NH{\cdot}COCH_3 + CH_3OH \longrightarrow C_6H_5NH_2 + CH_3COOCH_3 \qquad (8)$$

It is apparent from the hydrolysis procedure for nitriles (Chapter XIII) that the addition of 1 mole of water to nitriles to form the corresponding amides is relatively easy. Therefore, any technique for the hydrolysis of amides to the acids would also probably convert nitriles to the acids.

The effects of acids and bases were studied in experiments on the hydrolysis of amides and nitriles to the acids:

$$RCONH_2 + H_2O \longrightarrow RCOOH + NH_3 \qquad (9)$$

$$RCN + 2\,H_2O \longrightarrow RCOOH + NH_3 \qquad (10)$$

A quantitative procedure based on these reactions, in combination with the method for nitriles (Chapter XII), would permit the determination of amides and nitriles in the presence of other substances. Two series of experiments were tried using sulfuric acid. In the first, 5 milliequivalents of acetonitrile or adiponitrile in 5 ml. of acetic acid were treated with 5 ml. of concentrated sulfuric acid[2] (22 millimoles of water, 90 millimoles of acid), heating for 1 and 2 hours at 80°C. After 1 hour, 48 ± 5 per cent reaction was observed

---

[4] Hoog, H., and Eichwald, E., *Rec. trav. chim.*, 58, 481–92 (1939).

for both nitriles and, after 2 hours, 51 ± 3 per cent, based on complete hydrolysis to the acid. The data obviously indicated reaction to the amide only. In the second series, the same quantities of the nitriles were treated with 20 ml. of concentrated sulfuric acid and after heating at 80°C. suitable aliquots were titrated. The precision was extremely poor, the results varying between 40 and 58 per cent. Adipamide and acetamide under the same conditions did not react.[2]

During the course of a rather extensive study of the alkaline hydrolysis of nitriles and amides,[2] approximately 2 millimoles of acetamide with 20 ml. of 1 *N* sodium hydroxide in 98 per cent methanol were heated at 60°C. After 1 and 3 hours, respectively, only 11 and 27.5 per cent reaction was observed. In the most promising experiments, 4 milliequivalents of amide were weighed into 250 ml. glass-stoppered volumetric flasks. Ten milliliters of 1 *N* potassium hydroxide in essentially anhydrous diethylene glycol were added. (This reagent was recommended by Redemann and Lucas[5] for the saponification of esters.) Samples and blanks were placed in an oven at 125–128°C.

A 1 × 2 mm. groove was cut around the stoppers about 3 mm. above the bottom to permit a stopcock grease seal during the heating period. Prior to placing the samples in the oven the groove was filled with high-temperature stopcock grease and the stoppers were held in place with spring clamps.

After 1 to 3 hours at this temperature, the flasks were removed and allowed to cool to room temperature spontaneously. Five milliliters of glacial acetic acid were added, primarily to combine with the ammonia evolved, and the solutions were titrated with Karl Fischer reagent. Results are shown in Table CXLIII. In some cases known quantities of water were added to the amides before reaction.

The results of Table CXLIII indicated that essential complete reaction of formamide, acetamide, and adipamide was obtained after 1, 2, and 3 hours, respectively. The precision, however, was relatively poor. In order to study the reaction at higher temperatures an ampul was designed, shown in Figure 51a,[2] to allow heating in a sealed system. About 5 milliequivalent samples of acetamide, adipamide, acetonitrile, and adiponitrile were weighed into the ampuls. About 5 grams of 3.7 *N* potassium hydroxide in diethylene glycol were added. The stoppered tubes were cooled in

---

[5] Redemann, C. E., and Lucas, A. J., *Ind. Eng. Chem., Anal. Ed.*, **9**, 521-2 (1937).

TABLE CXLIII

Alkaline Hydrolysis of Amides[2]

Amide	Water added, mg.	Time at 128°C., hours	Per cent reaction
Formamide	0.0	1	98.5 ± 0.5
	66.2	1	99.7
	0.0	2	98.0 ± 0.5
Acetamide	0.0	1	95.5
	0.0	2	100.5 ± 0.1
	65.3	2	99.2
Adipamide	0.0	2	87.5
	0.0	3	96.5 ± 2.5
	64.0	3	90.0

dry ice and sealed off at the neck. Then they were heated for 30 minutes at 100°C. plus 2 hours at 180°C. After cooling, they were placed in a 500 ml. iodine flask, containing 26 ml. of glacial acetic acid, and were broken by pressing a glass rod against the thinner section of the ampul. The results varied between 38 and 55 per cent, based on hydrolysis of the amides to the acids.

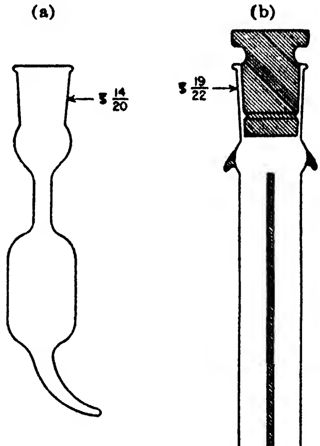


Fig. 51. (a) Ampul. (b) Modified Kraissel tube.

On the assumption that these low yields were due to unfavorable thermodynamic conditions (since any ammonia evolved tended to become a part of the vapor phase in the limited space above the liquid), a modified Kraissel drying tube was prepared (Fig. 51b[2]). In one experiment, acetamide with 7 ml. of 2 *N* potassium hydroxide in diethylene glycol was placed on one side of the partition and lauric acid, on the other. The acid was to serve as an ammonia absorber. After 2 hours at 130°C., the titration results indicated the reaction of acetamide to be 63 per cent complete.

A Fischer reagent procedure for amides probably could be developed which is based on their dehydration by 3,5-dinitrobenzoyl chloride.

Amides, like nitriles, normally have been identified by acid or alkaline hydrolysis. A specific procedure for the acidimetric determination of primary amides has been published, based on their quantitative dehydration with 3,5-dinitrobenzoyl chloride to the respective nitriles:[6]

$$\mathrm{RCONH} \xrightarrow{(\mathrm{NO_2})_2\mathrm{C_6H_3{\cdot}COCl}} \mathrm{RCN} + \mathrm{H_2O} \tag{11}$$

$$(\mathrm{NO_2})_2\mathrm{C_6H_3{\cdot}COCl} + \mathrm{H_2O} \longrightarrow (\mathrm{NO_2})_2\mathrm{C_6H_3{\cdot}COOH} + \mathrm{HCl} \tag{12}$$

Excess reagent was removed by reaction with dry methanol:

$$(\mathrm{NO_2})_2\mathrm{C_6H_3{\cdot}COCl} + \mathrm{CH_3OH} \longrightarrow (\mathrm{NO_2})_2\mathrm{C_6H_3{\cdot}COOCH_3} + \mathrm{HCl} \tag{13}$$

The net increase in acidity, after correction for water and free acid originally present in the sample, was equivalent to the primary amide originally present in the sample.

Such a method would be particularly valuable for amides in the presence of high concentrations of acids. Since the logical approach would involve hydrolysis of the excess acyl chloride after dehydration of the amide, such a method would require a stoichiometric correction for mixtures containing alcohols or amines; both of these types of compounds, in general, would be expected to react quantitatively with the 3,5-dinitrobenzoyl chloride.

Acylation techniques, for example, reaction with acetyl chloride, might be applicable to the estimation of phenols, the excess acyl halide (or anhydride) after reaction being determined by hydrolysis. A few experiments in the authors' laboratory[2] indicated about 90 per cent reaction of acetyl chloride with phenol, based on subsequent hydrolysis. This low result must have been due to experimental difficulties since phenols are known to react quantitatively by the acidimetric method.[7]

Another interesting possibility is the determination of tertiary in the presence of primary and secondary alcohols, based on their ability to dehydrate. For example, the reaction of *tert*-butanol would be:

$$(\mathrm{CH_3})_3\mathrm{COH} \longrightarrow (\mathrm{CH_3})_2\mathrm{C{=}CH_2} + \mathrm{H_2O} \tag{14}$$

A reagent of methanol, containing 100 grams of boron trifluoride per liter, dehydrated *tert*-butanol to the extent of 37 per cent after being heated for 2 hours at 60°C. (see Chapter IX).

Selective methods for acetals and ketals might be developed which are based on acid hydrolysis:

---

[6] Mitchell, J., Jr., and Ashby, C. E., *J. Am. Chem. Soc.*, 67, 161–4 (1945)

[7] Smith, D. M., and Bryant, W. M. D., *Ibid.*, 57, 61–5 (1935).

$$RCH(OR')_2 + H_2O \xrightarrow{H^+} RCHO + 2\,R'OH \qquad (15)$$

Similar reactions might be feasible for imines:

$$RCH{=}NR' + H_2O \xrightarrow{H^+} RCHO + R'NH_2 \qquad (16)$$

In both reactions (15) and (16), the aldehydes would have to be combined, e.g., as the cyanohydrins (page 151, Chapter V).

Xanthates, based on the ease with which they reduce iodine,[8,9] might be determined by direct titration with Fischer reagent:

$$2\,ROC(S)SNa + I_2 \longrightarrow 2\,NaI + [ROC(S)S]_2 \qquad (17)$$

Methods based on peroxide oxidation of thio ethers have been reported.[10]

$$RSR + H_2O_2 \longrightarrow RS(O)R + H_2O \qquad (18)$$

Since hydrogen peroxide does not interfere in the Karl Fischer titration, it should be possible to develop a technique in which the water formed, according to equation (18), could be related to thio ether concentration.

The above examples, of course, represent only a few of the potential applications of the Karl Fischer reagent. Almost any reaction which involves the quantitative or reproducible absorption or elimination of water probably could form the basis of an analytical procedure. It seems apparent that the analytical possibilities of the Karl Fischer reagent are just beginning to be realized.

---

[8] Whitmore, W. F., and Lieber, E., *Ind. Eng. Chem., Anal. Ed.*, **7**, 127–9 (1935).

[9] Sermais, B., *Rev. gén. mat. plastiques*, **12**, 165, 167–9 (1936).

[10] Toennies, G., and Callan, T. P., *J. Biol. Chem.*, **129**, 481–90 (1939)

AUTHOR INDEX

SUBJECT INDEX*

---

* Unless otherwise specified the index items refer to Karl Fischer procedures.